

Adrenal Hormone Secretion and Regulation of Adrenocortical Hypertrophy during Pregnancy in the Bank Vole, *Clethrionomys glareolus*

Bertil Andersson



Department of Zoology
University of Lund, Sweden

1983

We dance round in a ring and suppose,
But the Secret sits in the middle -
and knows

Robert Frost



ADRENAL HORMONE SECRETION AND REGULATION OF
ADRENOCORTICAL HYPERTROPHY DURING PREGNANCY
IN THE BANK VOLE, *CLETHRIONOMYS GLAREOLUS*

Doctoral thesis

by

Bertil Andersson

Fil kand, Hld

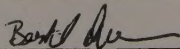
Akademisk avhandling som för avläggande av filosofie
doktorsexamen vid matematisk-naturvetenskapliga fakulteten
vid Lunds Universitet kommer att offentligens försvaras
å Zoologiska institutionen, Helgonavägen 3A, Lund,
fredagen den 3 juni 1983 kl 10.15

Organization LUND UNIVERSITY Department of Zoology Helgonavägen 3 S-223 62 Lund Sweden	Document name DOCTORAL DISSERTATION	
	Date of issue 1983-05-06	
	CODEN: LUNBDS/(NBZS-1013) 1-52/(1983)	
Author(s) Bertil Andersson	Sponsoring organization Swedish Natural Science Research Council	
Title and subtitle Adrenal Hormone Secretion and Regulation of Adrenocortical Hypertrophy during Pregnancy in the Bank Vole, <i>Clethrionomys glareolus</i>		
Abstract During pregnancy in the primiparous bank vole, <i>Clethrionomys glareolus</i>, there is a continuous increase in adrenal weight. This increase is seen regardless of how adrenal weight is expressed; either absolute or per unit of body weight. The adrenal enlargement is confined to the cortex. All three cortical zones increase in weight but zona fasciculata increases by far the most, comprising 75 % of the cortex on the day of parturition. After the first pregnancy the adrenals remain large only if the female is post-partum pregnant. The lactational status of the female has no effect on adrenal weight. Adrenal hypertrophy is also found during pseudopregnancy and in ovariectomized, mated females. This shows that mating triggers adrenal enlargement directly without involvement of ovarian hormones or any factors of foeto-placental origin. However, only prolonged mating, associated with increased prolactin levels will induce adrenal hypertrophy. Treatment with prolactin after limited mating causes adrenal enlargement, and inhibition of prolactin secretion (with pregnancy blockage or bromocriptine) partly blocks adrenal hypertrophy. Dexamethasone treatment, which blocks ACTH secretion, is also partly effective in hindering adrenal growth after mating. Thus, adrenal hypertrophy seems to be induced by a neuroendocrine mechanism triggered by complete mating and relying on at least prolactin and ACTH. During the second half of pregnancy there is a sharp increase in total corticosterone concentrations accompanied by a rise in CBG levels. During the last three days of pregnancy only CBG levels decline, presumably resulting in an increased level of unbound, biologically active corticosterone. As shown by the drop in corticosterone concentrations after adrenalectomy the adrenals are the most important source of this hormone. Progesterone appears to be only a minor adrenal secretion during pregnancy.		
Key words bank vole, adrenal cortex, pregnancy, mating, ACTH, prolactin, corticosterone, reproduction		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title		ISBN
Recipient's notes	Number of pages 52	Price
	Security classification	

Distribution by (name and address) Bertil Andersson, Department of Zoology, Helgonavägen 3, S-223 62 Lund, Sweden

I, the undersigned, being the copyright owner of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature



Date 1983-04-21

This thesis is mainly based on the following papers, which will be referred to by their roman numerals:

- I. Andersson, C.B. Adrenal hypertrophy during pregnancy in the bank vole, *Clethrionomys glareolus*: a morphometrical and histological study. (Manuscript). 15
- II. Gustafsson, T.O. & Andersson, C.B. Adrenal growth during pregnancy in the bank vole, *Clethrionomys glareolus*: initiation by mating. Can. J. Zool. 58: 1458-1461 (1980). 27
- III. Andersson, B. & Gustafsson, T. Relation between fertility and adrenal growth after mating in the bank vole, *Clethrionomys glareolus*. Can. J. Zool. 59: 329-331 (1981). 31
- IV. Andersson, C.B. & Gustafsson, T.O. Effect of limited and complete mating on ovaries and adrenals in bank voles, *Clethrionomys glareolus*. J. Reprod. Fert. 64: 431-435 (1982). 35
- V. Andersson, C.B. Effect of dexamethasone treatment on adrenal hypertrophy during pregnancy in the bank vole, *Clethrionomys glareolus*. (Manuscript). 41
- VI. Andersson, C.B. & Vawda, A. Plasma corticosterone and corticosteroid-binding globulin concentrations during pregnancy and pseudo-pregnancy in the bank vole, *Clethrionomys glareolus*. (submitted for publication). 45

INTRODUCTION

The bank vole, *Clethrionomys glareolus*, is a cricetid rodent of the subfamily Microtinae. The majority of microtine species are found in North America, Europe and northern Asia. The geological range of the family *Cricetidae* in this area is from the Oligocene to the Recent (Walker, 1964). The five species of *Clethrionomys* live in a wide variety of habitats - forest, woodland, tundra and bog. They have a heavy-set body (7-10 cm), blunt rounded muzzle, short legs and a short tail (less than half the body length). The colouration above is greyish-brown with a reddish wash, greyish flanks and grey to white underparts. *Clethrionomys* are active day and night, summer and winter, feeding on tender vegetation, seeds, bark, lichens, fungi and insects. They do not burrow, but nest in crevices lined with shredded vegetation. When startled they utter a high-pitched chirp and may also chatter their teeth. Most individuals live less than a year in the wild as they are important prey for carnivores and birds of prey.

Bank voles have a restricted breeding season lasting from early spring until late autumn (Nyholm & Meurling, 1979). Several litters are born during the breeding season and the offspring may breed during their season of birth. However, reproductive parameters are dependent on population density (Nyholm & Meurling, 1979). Remarkable fluctuations in numbers are observed in many microtine populations. Northern populations show a regular cyclic periodicity (3 to 4 years) while southern ones are almost stable (Nyholm & Meurling, 1979; Wiger, 1979).

In both stable and cyclic populations of several microtine species the adrenal glands of the females enlarge during the breeding season (see I). The functional relations between adrenal growth and the reproductive state of the female are, however, not clarified. The main cause for this is the difficulty in making a correct determination of the reproductive state of field trapped females. This, and many other, difficulties are overcome by using laboratory bred animals.

The aim of this thesis has been to describe in some detail the adrenal enlargement of female bank voles and to study the causes for and the consequences of this hypertrophy.

RESULTS AND GENERAL DISCUSSION

Adrenal hypertrophy (I)

During pregnancy in the primiparous bank vole there is a continuous increase in adrenal weight. This increase is seen regardless of how adrenal weight is expressed; either absolute or per unit of body weight. After the first pregnancy the adrenals remain large only if the females are post-partum pregnant. The lactational status of the female has no effect on adrenal weight.

The physiological variation among the females used in paper I was kept to a minimum at the start of the experiment. They were all 2-4 months old, primiparous and kept under similar, controlled conditions (Gustafsson, Andersson & Westlin, 1980). It seems reasonable to argue that the only important difference between the females was their different stage of pregnancy and/or lactation.

It appears that the adrenal weight increase during the breeding season observed in field samples (Chitty, 1961; Christian & Davis, 1966; Sealander, 1967; Valentine & Kirkpatrick, 1970; Andersson, Gustafsson, Meurling & Nyholm, 1978) is caused by the enlargement of the adrenals of pregnant females.

The adrenal enlargement is confined to the cortex. The cortical zones all increase in weight but zona fasciculata increases by far the most, coming to occupy 75 % of the cortex on the day of parturition. After pregnancy there is a decrease in weight of all three zones.

Compared with other microtines there are several differences in the behaviour of the bank vole adrenals during pregnancy. The weight increase is much more pronounced than in e.g. the field vole and is caused by the enlargement of a different zone, z. fasciculata, rather than the juxtamedullary zone (Chitty & Clarke, 1963; Jorné-Safriel, 1968). Moreover, the juxtamedullary zone of the adrenal cortex of the bank vole bears a stronger resemblance to a normal z. reticularis than to an X-zone, as the juxtamedullary zone has been labeled by several investigators (Delost & Delost, 1954; Chitty & Clarke, 1963; Tähkä, 1979). The correspondence between z. reticularis and the X-zone in some microtines has been noted earlier by Christian & Davis (1966) and Siuda (1973).

Regulation of adrenal hypertrophy (II, III, IV, V and unpublished)

The growth of the adrenals during pregnancy - a time of intense, pituitary-regulated ovarian hormone secretion and growth of the fetus - implies a connection between these tissues. However, the finding that the adrenal weight increases during pseudopregnancy (I), i.e. a period of luteal activity without conceptuses, shows that the presence of embryos and placentae is not a prerequisite for pregnancy-associated adrenal growth. The work was therefore concentrated on the importance of the ovaries and the pituitary for adrenal enlargement.

The bank vole is a reflex ovulator (Clarke, Clulow & Greig, 1970) and shows no regular oestrus cyclicity (Andersson, unpublished). This seems to be the rule among microtines (see Milligan, 1982 for review). Thus, it is haphazardous to obtain mated bank voles. Receptivity can, however, be achieved in females by oestradiol treatment. This method was used to study the role of ovarian hormones in adrenal growth after mating (II). The weight of the adrenals in ovariectomized, oestradiol treated and mated females was compared with that in ovariectomized, oestradiol treated and unmated ones. After four days the mated females

had significantly heavier adrenals than the unmated ones which in turn did not differ in weight from those of untreated and unmated animals of similar age. This experiment shows that oestradiol treatment does not cause adrenal growth and that mating causes adrenal hypertrophy directly without involvement of ovarian hormones.

Bearing in mind that neither embryos nor ovaries seem to be necessary for the triggering of adrenal growth it was unexpected to find a relationship between fertility and adrenal growth (III). Fertility is low in primiparous microtines (Coutts & Rowlands, 1969; Milligan & MacKinnon, 1976; Andersson & Gustafsson, 1979). In bank voles 50-75 % of the mature females become pregnant after their first mating (III; Andersson, unpublished). The pregnant females have heavier adrenals than the non-pregnant ones.

An explanation to the low fertility and the difference in adrenal weight may be found in the mechanism for ovulation and activation of the corpora lutea (CL). In the field vole ovulation is caused by an LH-surge induced by the first few intromissions but this gives rise only to short-lived CL (Milligan, 1975; Milligan & MacKinnon, 1976). More prolonged mating results in the formation of functional CL because of elevated prolactin levels (Milligan & MacKinnon, 1976). If for some reason the stimulation requirements for CL activation are not met at mating or an inadequacy exists in the neuroendocrine pathways of primiparous females (Westlin & Gustafsson, 1983), the low fertility could be explained. Furthermore, if functional CL and adrenal growth are triggered by the same mechanism the relationship between fertility and adrenal growth is accounted for.

The possibility to distinguish separate neuroendocrine events after mating by providing different amounts of mating stimulation gave rise to the following experiment (IV). Female bank voles were allowed either a complete mating (several hours) or only limited mating (i.e. 1-3 intromissions, a few minutes). Both treatments induced ovulation but active CL and adrenal hypertrophy were only found after a complete mating. If limited mating was followed by mechanical genital stimulation, active CL and adrenal hypertrophy were induced. This shows that the induction of adrenal growth, like activation of the CL, requires greater amounts of mating than ovulation and is accomplished only after prolonged mating, associated with increased prolactin levels. Apparently LH alone does not cause adrenal enlargement. It seems that prolactin, alone or in combination with LH, is part of the neuroendocrine reflex inducing adrenal hypertrophy after mating.

The possible role of prolactin was investigated in three ways: by studying the effect of pregnancy block on the adrenals, by bromocriptine (a dopamine agonist) treatment and by prolactin treatment. If newly mated females of many rodent species are exposed to a strange male a block to pregnancy results (Bruce, 1959; Clulow & Clarke, 1968; Clarke & Clulow, 1973). The immediate cause is a failure of CL function, resulting in a failure of implantation (Bruce, 1960; Parkes & Bruce, 1961; Milligan, 1976). The reason for this luteal failure has been suggested to be a suppression of prolactin (Bruce & Parkes, 1960; Milligan, 1976).

In my experiment on pregnancy block, twenty female bank voles were mated and left with the stud male for 48 hours. They were then housed with a strange male for 10 hours. Six days after mating two females were pregnant; the remaining 18 were non-pregnant with one set of regressing CL. Adrenal weight was significantly lower in the females exhibiting pregnancy block (8.03 ± 0.57 mg) than in 6 day pregnant females (10.55 ± 0.86 mg; $P < 0.02$).

The importance of prolactin for adrenal hypertrophy is further supported by the results of bromocriptine treatment, which inhibits prolactin secretion (Flückiger, 1978; Charlton, Milligan & Versi, 1978). Bromocriptine (1 mg in 0.1 ml injections; 10 mg dissolved in 0.5 ml 70 % alcohol and 0.5 ml saline containing 10 mg tartaric acid) was given subcutaneously after 1-3 intromissions whereafter mating was allowed to continue. Six days later adrenals were lighter in treated females (7.99 ± 0.40 mg; $n = 7$) than in untreated pregnant females (10.55 ± 0.86 ; $n = 12$; $0.05 < P < 0.10$). Twelve days after mating (an additional 1 mg of bromocriptine was given on day 6) adrenal weight was 7.62 ± 1.21 mg ($n = 6$) compared with 16.46 ± 2.33 mg ($n = 6$) in pregnant females ($P < 0.01$). All bromocriptine treated females were non-pregnant and had regressing CL except for one which was pregnant. Injections of bromocriptine vehicle had no effect on pregnancy or adrenal weight on day 12 (15.10 ± 1.23 mg; $n = 3$).

Prolactin was given to eleven females after limited mating (10 iu daily, subcutaneously in 0.1 ml saline). Twelve days after limited mating the mean adrenal weight was 7.91 ± 0.61 mg, significantly higher than in untreated virgins (5.72 ± 0.78 mg; $n = 5$; $P < 0.05$), but lower than in untreated pregnant females (16.46 ± 2.33 mg; $n = 6$).

The different experiments on the role of prolactin all support the idea that prolactin is involved in, but is not the sole cause for, adrenal hypertrophy after mating.

An obvious hormone to study in connection with adrenal hypertrophy is ACTH. This was done by dexamethasone treatment which blocks ACTH secretion (V). Mated females injected with dexamethasone had lighter adrenals on day 12 of pregnancy than did females receiving vehicle only. The adrenal weight of the controls did not differ from that of untreated pregnant females. It appears that ACTH, in addition to prolactin, is important to adrenal hypertrophy during pregnancy.

Adrenal hormone secretion (VI and unpublished)

The adrenals as an additional source of progesterone was investigated. This was done in view of the findings of progesterone secretion by the rat adrenals during the oestrous cycle (Feder, Resko & Goy, 1968; Piva, Gagliano, Motta & Martini, 1973; Shaikh & Shaikh, 1975) and the regulation of adrenal progesterone by ACTH and prolactin (Resko, 1969; Piva et al., 1973; Mann, Cost, Jacobson & MacFarland, 1977; Ogle & Kitay, 1979).

Female bank voles were treated as shown in Table 1. Plasma progesterone was measured by radioimmunoassay, using the method of Milligan, Charlton & Versi (1979). It is obvious from Table 1 that the sources of progesterone are

the ovaries and the adrenals and that the completely dominating progesterone source during pregnancy are the ovaries. As expected the adrenals of non-pregnant females were lighter than those of pregnant ones which in turn did not differ in adrenal weight from ovariectomized animals (II, III). Thus, the low progesterone concentrations in the ovariectomized females can not be explained by a lesser amount of adrenal tissue.

Table 1. Plasma progesterone concentrations and paired adrenal weights (mean $\pm$ S.E.) 6 days after mating in pregnant and non-pregnant bank voles. ADX = adrenalectomized, OVX = ovariectomized, UD = undetectable

Treatment after mating	Progesterone ng/ml		Adrenals mg		Proportion of pregnant voles
	preg.	non-preg.	preg.	non-preg.	
Intact	64.9 $\pm$ 5.2	14.7 $\pm$ 1.8	9.28 $\pm$ 0.70	6.50 $\pm$ 0.75	12/15
ADX	65.3 $\pm$ 9.6	11.5 $\pm$ 2.6	-	-	4/15
OVX	-	9.7 $\pm$ 3.0	-	9.23 $\pm$ 1.20	0/8
ADX+OVX	-	UD	-	-	0/12

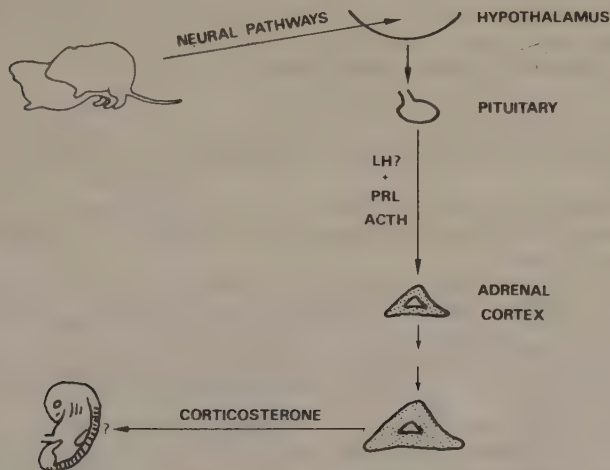
Another important adrenal hormone is corticosterone, mainly secreted by zona fasciculata (Idelman, 1978). Since z. fasciculata is the largest zone during pregnancy in the bank vole (I) it was of interest to determine corticosterone concentrations (VI). We found that during the second half of pregnancy there is a sharp increase in total corticosterone concentrations accompanied by a rise in corticosteroid-binding globulin (CBG) levels. During the last three days of pregnancy CBG concentrations decline while corticosterone levels remain high. This is likely to result in an increased level of unbound, biologically active corticosterone. As shown by the drop in corticosterone concentrations after adrenalectomy the adrenals are the most important source of this hormone.

Few suggestions to the biological significance of high corticosterone levels during pregnancy have been offered. It has been speculated that corticosterone may induce lactogenesis in the rat (Gala & Westphal, 1967) but conclusive evidence is lacking.

Only circumstantial evidence of any importance of corticosterone during pregnancy is available for the bank vole. In dexamethasone treated pregnant bank voles 6 out of 8 females had resorbing embryos on day 12 of pregnancy (V). This can be caused either by a direct adverse effect of dexamethasone on the embryos or by a lack of an ACTH-dependent adrenal secretion, probably corticosterone. On the other hand, adrenalectomy on day 15 of pregnancy had no effect on the embryos (VI). When newly mated females were adrenalectomized, only 4 out of 15 were pregnant 6 days later. This fertility rate is lower than expected (discussed above) and can be due to the removal of adrenal hormones or simply to operational stress affecting the activation of CL.

At present it is obviously not possible to offer any explanation of the role of corticosterone during pregnancy in the bank vole.

The findings in this thesis may be summarized in the following schematical way:



ACKNOWLEDGEMENTS

I am indebted to Prof. em. Erik Dahl and to Prof. Rolf Elofsson for support and laboratory facilities. Further, I would like to thank Doc. Patrick Meurling and Doc. Erik Nyholm who initiated my interest in reproductive endocrinology. I am especially grateful to my friend and co-worker Dr. Torgny Gustafsson without whom there would have been nothing but a start. Dr. Lilian Westlin provided help at various times and for this and a fruitful friendship I thank her.

I would also like to express my thanks to the entire scientific and technical staff at the Department for help, friendship and a lot of fun through the years.

The hormone analyses were carried out at King's College, London. I would like to express my gratitude to Dr. Stuart Milligan for his generous help and for the many inspiring discussions.

My sincere thanks are also due to my wife, Birgitta, for her patience with a preoccupied husband with all but regular working hours.

Financial support is acknowledged from the Swedish Natural Science Research Council and the Royal Physiographic Society of Lund.

REFERENCES

- Andersson, C.B. & Gustafsson, T.O. (1979) Delayed implantation in lactating bank voles, *Clethrionomys glareolus*. J. Reprod. Fert. 57: 349-352.
- Andersson, B., Gustafsson, T., Meurling, P. & Nyholm, E. (1978) The role of the adrenal cortex in reproduction in the female bank vole, *Clethrionomys glareolus*. In: Comparative Endocrinology, eds. P.J. Gaillard & H.H. Boer. Elsevier/North-Holland. p. 83.
- Bruce, H.M. (1959) An exteroceptive block to pregnancy in the mouse. Nature 184: 105.
- Bruce, H.M. (1960) A block to pregnancy in the mouse caused by proximity of strange males. J. Reprod. Fert. 1: 96-103.
- Bruce, H.M. & Parkes, A.S. (1960) Hormonal factors in exteroceptive block to pregnancy in mice. J. Endocrinol. 20: xxix-xxx.
- Charlton, H.M., Milligan, S.R. & Versi, E. (1978) Studies on the control of the corpus luteum in the vole, *Microtus agrestis*. J. Reprod. Fert. 52: 283-288.
- Chitty, H. (1961) Variations in the weight of the adrenal glands of the field vole, *Microtus agrestis*. J. Endocrinol. 22: 387-393.
- Chitty, H. & Clarke, J.R. (1963) The growth of the adrenal glands of laboratory and field voles, and changes in it during pregnancy. Can. J. Zool. 41: 1025-1034.
- Christian, J.J. & Davis, D.E. (1966) Adrenal glands in female voles (*Microtus pennsylvanicus*) as related to reproduction. J. Mammal. 47: 1-18.
- Clarke, J.R. & Clulow, F.W. (1973) Effect of successive matings upon bank vole, *Clethrionomys glareolus*, and vole, *Microtus agrestis*, ovaries. In: The Development and Maturation of the Ovary and its Functions, ed H. Peters. Excerpta medica I.C.S. No. 267, Amsterdam. pp. 160-170.
- Clarke, J.R., Clulow, F.W. & Greig, F. (1970) Ovulation in the bank vole, *Clethrionomys glareolus*. J. Reprod. Fert. 23: 531.
- Clulow, F.W. & Clarke, J.R. (1968) Pregnancy-block in *Microtus agrestis*, an induced ovulator. Nature 219: 511.
- Coutts, R.R. & Rowlands, I.W. (1969) The reproductive cycle of the Skomer vole (*Clethrionomys glareolus skomerensis*). J. Zool., Lond. 158: 1-25.
- Delost, P. & Delost, H. (1954) Existence d'une zone X sur-rénalienne chez le Campagnol roussâtre (*Clethrionomys glareolus* S.). C. R. Soc. Biol. 148: 1788-1791.
- Feder, H.H., Resko, J.A. & Goy, R.W. (1968) Progesterone levels in the arterial plasma of pre-ovulatory and ovariectomized rats. J. Endocrinol. 41: 563-569.
- Flückiger, E. (1978) Effects of bromocriptine on the hypothalamo-pituitary axis. Acta Endocrinol. 88: 111-117.
- Gala, R.R. & Westphal, U. (1967) Corticosteroid-binding activity in serum of mouse, rabbit and guinea pig during pregnancy and lactation: possible involvement in the initiation of lactation. Acta Endocrinol. 55: 47-61.

- Gustafsson, T., Andersson, B. & Westlin, L. (1980) Reproduction in a laboratory colony of bank vole, *Clethrionomys glareolus*. Can. J. Zool. 58: 1016-1021.
- Idelman, S. (1978) The structure of the mammalian adrenal cortex. In: General, Comparative and Clinical Endocrinology of the Adrenal Cortex, eds. I. Chester Jones & I.W. Henderson. Vol. 2. Academic Press.
- Jorné-Safriel, O. (1968) Some factors affecting the adrenal juxtamedullary zone in the vole, *Microtus agrestis*, and bank vole, *Clethrionomys glareolus*. D. Phil. thesis, University of Oxford.
- Mann, D.R., Cost, M.G., Jacobson, C.D. & MacFarland, L.A. (1977) Adrenal gland rhythmicity and pituitary regulation of adrenal steroid secretion. Proc. Soc. Exp. Biol. Med. 156: 441-445.
- Milligan, S.R. (1975) Mating, ovulation and corpus luteum function in the vole, *Microtus agrestis*. J. Reprod. Fert. 42: 35-44.
- Milligan, S.R. (1976) Pregnancy block in the vole (*Microtus agrestis*). II. Ovarian, uterine and vaginal changes. J. Reprod. Fert. 46: 97-100.
- Milligan, S.R. (1982) Induced ovulation in mammals. In: Oxford Reviews of Reproductive Biology, ed. C.A. Finn. Vol. 4. Oxford University Press. pp. 1-46.
- Milligan, S.R., Charlton, H.M. & Versi, E. (1979) Evidence for a coitally induced "mnemonic" involved in luteal function in the vole (*Microtus agrestis*). J. Reprod. Fert. 57: 227-233.
- Milligan, S.R. & MacKinnon, P.C.B. (1976) Correlation of plasma LH and prolactin levels with the fate of the corpus luteum in the vole, *Microtus agrestis*. J. Reprod. Fert. 47: 111-113.
- Nyholm, N.E.I. & Meurling, P. (1979) Reproduction in the bank vole, *Clethrionomys glareolus*, in northern and southern Sweden during several seasons and in different phases of the vole population cycle. Holarctic Ecol. 2: 12-20.
- Ogle, T.F. & Kitay, J.I. (1979) Interactions of prolactin and adrenocorticotropin in the regulation of adrenocortical secretions in female rats. Endocrinol. 104: 40-44.
- Parkes, A.S. & Bruce, H.M. (1961) Olfactory stimuli in mammalian reproduction. Science 134: 1049-1054.
- Piva, F., Gagliano, P., Motta, M. & Martini, L. (1973) Adrenal progesterone: factors controlling its secretion. Endocrinol. 93: 1178-1184.
- Resko, J.A. (1969) Endocrine control of adrenal progesterone secretion in the ovariectomized rat. Science 164: 70-71.
- Sealand, J.A. (1967) Reproductive status and adrenal size in the northern red-backed vole in relation to season. Int. J. Biometeorol. 11: 213-220.
- Shaikh, A.A. & Shaikh, S.A. (1975) Adrenal and ovarian steroid secretion in the rat estrous cycle temporally related to gonadotropins and steroid levels found in peripheral plasma. Endocrinol. 96: 37-44.
- Siuda, S. (1973) The occurrence of X-zone in the adrenal glands of two rodent species. Acta theriol. 18: 471-479.

- Tähkä, K.M. (1979) Age and sex differences in the distribution of lipids, tetrazolium reductases and hydroxysteroid dehydrogenase in the gonads and adrenals of the bank vole (*Clethrionomys glareolus*): a histochemical study. Biol. Reprod. 21: 125-133.
- Valentine, G.L. & Kirkpatrick, R.O. (1970) Seasonal changes in reproductive and related organs in the pine vole, *Microtus pinetorum*, in southwestern Virginia. J. Mammal. 51: 553-560.
- Walker, E.P. (1964) Mammals of the World. Vol. II. The Johns Hopkins Press, Baltimore.
- Westlin, L.M. & Gustafsson, T.O. (1983) Influence of sexual experience and social environment on fertility and incidence of mating in young female *Clethrionomys glareolus*. J. Reprod. Fert. in press.
- Wiger, R. (1979) Demography of a cyclic population of the bank vole, *Clethrionomys glareolus*. Oikos 33: 373-385.

ADRENAL HYPERTROPHY DURING PREGNANCY IN THE BANK VOLE, *CLETHRIONOMYS GLAREOLUS*: A MORPHOMETRICAL AND HISTOLOGICAL STUDY

C. Bertil Andersson

Department of Zoology, University of Lund,
S-223 62 Lund, Sweden

Summary. The adrenal weight (absolute and relative) of female bank voles increases during pregnancy and pseudopregnancy. The increase is evident already a few days after mating and continues throughout pregnancy (5.7 mg on day 0 to 21.7 mg on day 18). During pseudopregnancy the adrenal weight is about doubled (4.8 mg on day 0 to 8.1 mg on day 10). Only the cortex increases in weight, the heaviest zone being the zona fasciculata, occupying 75 % of the cortex on day 18. The lactational state of the female has no influence on the adrenal weight.

INTRODUCTION

From several field studies of microtines it is evident that the weight of the adrenal glands of females increases during the breeding season. This has been found in *Microtus agrestis* (Chitty, 1961; Pankakoski & Tähkä, 1982), in *M. pennsylvanicus* (Christian & Davis, 1966), in *M. pinetorum* (Valentine & Kirkpatrick, 1970), in *Clethrionomys rutilus* (Sealand, 1967) and in *C. glareolus* (Andersson, Gustafsson, Meurling & Nyholm, 1978, see also Kime, Vinson, Major & Kilpatrick, 1980; Pankakoski & Tähkä, 1982). However, it is not clear in what way this weight increase relates to the reproductive state of the female. The present investigation was undertaken to study the adrenal glands of bank voles under controlled conditions during pregnancy, pseudopregnancy and lactation.

MATERIALS AND METHODS

The laboratory bred bank voles, *Clethrionomys glareolus*, used in this study are descendants of wild voles from a stable population in southern Sweden (Nyholm & Meurling, 1979). They were kept as described by Gustafsson, Andersson & Westlin (1980). From weaning at 18 days until the beginning of the experiment at 2-4 months of age the females were kept in groups of 4-5. They were then caged singly for 1-2 days before an adult male - intact or vasectomized - was introduced to each female in the morning. The pair was checked every 1-2 h to see if mating had occurred. After an observed mating or the finding of a vaginal plug, the male was left with the female overnight. If no mating occurred during the day, the male was removed and introduced again the following morning. The day of mating was designated day 0 of pregnancy or pseudopregnancy (resulting from a

mating with a vasectomized male). Females were killed at various times during pregnancy, pseudopregnancy and lactation (see Figs. 1 & 2). At autopsy reproductive organs and adrenals were dissected out, weighed fresh to an accuracy of 0.1 mg. Total body weights were recorded to an accuracy of 0.1 g. The left adrenal was fixed in Bouin's fluid, embedded in paraffin wax, serially sectioned (7 μ m) and stained with Ehrlich's haematoxylin and eosin. All sections were perpendicular to the long axis of the adrenal. The section with the greatest area was selected with an eyepiece micrometer. With the aid of a camera lucida the outline of the whole cortex and its three zones and the medulla of this section was traced. The width of the cortex and its zones were measured at points chosen at random. A transparent sheet was superimposed upon each tracing of adrenal sections. From the center of the sheet (coinciding with the center of the medulla) 8 numbered lines radiated. A random generator was used to choose 4 lines along which the widths of zones were measured. The mean of the 4 measurements of each zone was used as the width of that zone. Weights of the zones of the cortex and of the medulla were calculated according to Chumachenko (1977), using the equation

$$W_x = (W_{\text{tot}} - \sum_{n=1}^3 W_{x-n}) \times \frac{(R - \sum_{n=1}^3 L_{x-n})^3 - (R - \sum_{n=0}^2 L_{x-n})^3}{(R - \sum_{n=1}^3 L_{x-n})^3}$$

where

W_{tot} = weight of adrenal gland

R = width of cortex

W_1, L_1 = weight and width of zona glomerulosa, respectively

W_2, L_2 = weight and width of zona fasciculata, respectively

W_3, L_3 = weight and width of zona reticularis, respectively

W_4, L_4 = weight and width of medulla, respectively

Terms with an index ≤ 0 are not defined.

Data were analysed using the Mann-Whitney U-test and analysis of variance, where appropriate. All tests were two-tailed.

RESULTS

The mean paired adrenal weights of 97 pregnant bank voles are given in Fig. 1. No difference in adrenal weight was found between virgins and newly mated females of similar age. As pregnancy continued the adrenal weight increased. The increase had started already 24 h after mating and 3 days after mating the adrenals of pregnant females were significantly heavier than those of newly mated females ($P < 0.01$). The adrenal weight increase continued up to day 14 and was then followed by an insignificant decrease. Thereafter a significant increase was found from day 15 to day 18 ($P < 0.01$).

A similar adrenal weight increase during pregnancy was found when relative adrenal weight (adrenal weight/body weight) was calculated (Table 1). Even if conceptuses were included in the body weight of the mother, relative adrenal weight was significantly higher on days 3-18 than on day 0.

After pregnancy, the adrenals decreased in weight if the female was not mated post-partum (Table 2). Three days after parturition adrenal weight was significantly reduced compared with day 18 of pregnancy ($P < 0.002$; Fig. 1). Adrenals remained large in post-partum pregnant females. The lactational status of the female had no effect on adrenal weight (Table 3).

During the first 3 days of pseudopregnancy there was a significant increase in absolute adrenal weight ($P < 0.02$; Fig. 2). The adrenals of pseudopregnant females were as heavy as those of pregnant ones except on day 10 ($P < 0.02$). The elevated level was maintained throughout pseudopregnancy. The return to non-pseudopregnant values was slow; 18 days after mating with a vasectomized male adrenal weight was still high (8.38 ± 1.13 mg).

The adrenal cortex increased in weight during pregnancy and pseudopregnancy, while no significant changes in the weight of the medulla were found (Figs. 1 & 2). The heaviest cortical zone during pregnancy was zona fasciculata. It was heavier than any other zone on day 3 ($P < 0.02$) and on days 12, 15 and 18 ($P < 0.01$). The weight increase of z. fasciculata was quite parallel to that of the whole gland. On days 6-18 it was significantly heavier than on day 0 ($P < 0.01 - 0.002$). After pregnancy the weight of z. fasciculata decreased significantly during the first 3 days of lactation ($P < 0.01$). Z. reticularis did also increase in weight during pregnancy. On days 9-18 it was significantly heavier than on day 0 ($P < 0.05 - 0.01$). Z. glomerulosa showed a small weight increase during pregnancy and on day 18 it was significantly different from day 0 ($P < 0.02$).

A similar relationship between the weight changes of the zones of the cortex was found during pseudopregnancy (Fig. 2). However, no changes were significant. This was probably mainly due to a too small sample on day 0.

The histology of z. glomerulosa did not change during pregnancy. The cells, in ovoid groups, had round nuclei with one or two nucleoli and a lightly coloured cytoplasm. The polyhedral cells of the z. fasciculata, arranged in radial rows, increased in size during pregnancy (Plate 1). Two types of cells were distinguished; one with deeply eosinophilic cytoplasm and one lightly coloured. The two cell types were intermingled and not separated in layers. Both had round nuclei with one or two nucleoli. Lipid droplets were abundant in the cytoplasm.

The reticular cells were smaller than those of z. fasciculata, although they increased in size during the first half of pregnancy. In a few animals the cells closest to the medulla were larger than the others. The colour of the cytoplasm was intermediate between the dark and light fascicular cells. The spherical nuclei were as basophilic as the nuclei of z. fasciculata. The cells had fine lipid droplets, but

these were much smaller and not as common as in *z. fasciculata*. The networks of reticular cells surrounded large blood sinuses. Only little connective tissue was found surrounding the medulla.

DISCUSSION

The present study shows that the weight of the adrenal glands increased during pregnancy in the bank vole. An increase was found both in absolute and relative adrenal weight. This is in keeping with earlier observations (Jorné-Safriel, 1968) and agrees well with the finding of heavier adrenals in pregnant than in non-pregnant field trapped animals (Delost & Delost, 1954).

Also in naturally occurring populations of field voles the adrenals of pregnant animals were found to be heavier than those of nulliparous ones (Chitty, 1961) and laboratory studies showed an adrenal weight increase during pregnancy (Chitty & Clarke, 1963; Jorné-Safriel, 1968). In a study of laboratory bred collared lemmings, *Dicrostonyx groenlandicus*, Hasler & Banks (1975) found a positive correlation between adrenal growth and pregnancy. In spite of these results there is some dispute on whether an adrenal enlargement during pregnancy occurs in microtines or not, since conflicting data have been obtained. McKeever (1959) reported that the adrenals of pregnant *M. montanus* were not larger than those of non-pregnant but "sexually active" animals. No difference in mean adrenal weight between pregnant and non-pregnant *M. pennsylvanicus* was found by Christian & Davis (1966). More recently Pankakoski & Tähkä (1982) stated that adrenals of pregnant field voles and bank voles were not heavier than those of other mature females. The contradictory results obtained in these three field studies are most likely due to a large number of the females having been classified as non-pregnant when they were in fact pregnant, either early (before implantation) in their first pregnancy or early post-partum pregnant. Furthermore, since the return to non-pregnant adrenal weight levels after parturition is slow, non-pregnant females either lactating or non-lactating would exhibit enlarged adrenals.

The adrenal weight increase during pseudopregnancy found here confirms previous results in bank voles and field voles (Jorné-Safriel, 1968) and in collared lemmings (Hasler & Banks, 1975). It is obvious that the changes in the adrenal glands are independent of implantation.

During lactation Jorné-Safriel (1968) found progressively lighter adrenals in non-pregnant field voles and bank voles. This is in agreement with the findings in this study. Lactation did not support adrenal hypertrophy; it is only found if the female is post-partum pregnant. However, the rate of adrenal weight decline found here was slower than that found by Jorné-Safriel (1968) and in contrast few degenerative changes were seen in the cortical cells.

The adrenal weight increase during pregnancy and pseudopregnancy in the bank vole is due to an increased amount of cortex. No weight increase of the medulla was found.

Adrenocortical enlargement during pregnancy has been described in several microtines (Chitty & Clarke, 1963; Christian & Davis, 1966; Jorné-Safriel, 1968). The part of the cortex most often said to increase is the innermost zone. In this study, however, the largest weight increase was shown by the z. fasciculata. The juxtamedullary portion of the cortex did also enlarge but less so than z. fasciculata. Jorné-Safriel (1968) found that the juxtamedullary part came to occupy 55 % of the cortex. In the present study its maximum was 32 % of the cortical weight, while z. fasciculata occupied a maximum of 75 %.

As in *M. pennsylvanicus* (Christian & Davis, 1966) and in *C. glareolus* (Siuda, 1973) no reasons for labeling the innermost cortical zone an X-zone were found. It did not fulfill the criteria for an X-zone originally set by Howard-Miller (1927) as a zone of the mouse adrenal with "specific variations under certain conditions". The juxtamedullary portion of the bank vole adrenal cortex did not behave as that of the mouse. Furthermore, histologically it resembled a normal z. reticularis more than an X-zone.

The changes in the adrenal glands of pregnant bank voles seem to differ somewhat from those reported in other microtines. The weight increase of the gland is much more dramatic and a different cortical zone enlarges compared with the field vole (Chitty & Clarke, 1963). This is not caused by the different methods employed (zone weight and zone area) since the differences remain when the data from Chitty & Clarke (1963) are transformed to zone weights. Thus, there appears to exist species differences in adrenal changes during pregnancy in two closely related voles. Further studies are needed before the significance of these differences can be explained.

This work was supported by grants from the Swedish Natural Science Research Council and the Royal Physiographic Society of Lund. I am indebted to Mrs G. Behm for expert technical assistance.

REFERENCES

- Andersson, B., Gustafsson, T., Meurling, P. & Nyholm, E. (1978). The role of the adrenal cortex in reproduction in the female bank vole, *Clethrionomys glareolus*. p. 83. In: Comparative Endocrinology. Eds. P.J. Gaillard & H.H. Boer. Elsevier/North-Holland.
- Chitty, H. (1961). Variations in the weight of the adrenal glands of the field vole, *Microtus agrestis*. J. Endocrinol. 22, 387-393.
- Chitty, H. & Clarke, J.R. (1963). The growth of the adrenal glands of laboratory and field voles, and changes in it during pregnancy. Can. J. Zool. 41, 1025-1034.
- Christian, J.J. & Davis, D.E. (1966). Adrenal glands in female voles (*Microtus pennsylvanicus*) as related to reproduction. J. Mammal. 47, 1-18.

- Chumachenko, P.A. (1977). Morphometry of the adrenals. Bull. exp. Biol. Med. 83, 751-754.
- Delost, P. & Delost, H. (1954). Existence d'une zone X sur-rénalienne chez le Campagnol roussâtre (*Clethrionomys glareolus* S.). C. R. Soc. Biol. 148, 1788-1791.
- Delost, P. & Delost, H. (1955). Modifications pondérales et histologique des glandes surrénales du Campagnol agreste (*Microtus agrestis* L.) en fonction de l'état sexuel. C. R. Soc. Biol. 149, 910-914.
- Gustafsson, T., Andersson, B. & Westlin, L. (1980). Reproduction in a laboratory colony of bank vole, *Clethrionomys glareolus*. Can. J. Zool. 58, 1016-1021.
- Hasler, J.F. & Banks, E.M. (1975). Morphological changes in the ovaries and other organs of the collared lemming (*Dicrostonyx groenlandicus*) during pregnancy and pseudo-pregnancy. Can. J. Zool. 53, 12-18.
- Howard-Miller, E. (1927). A transitory zone in the adrenal cortex which shows age and sex relationships. Amer. J. Anat. 40, 251-278.
- Jorné-Safriel, O. (1968). Some factors affecting the adrenal juxtamedullary zone in the vole, *Microtus agrestis*, and bank vole, *Clethrionomys glareolus*. D. Phil. Thesis, University of Oxford.
- Kime, D.E., Vinson, G.P., Major, P.W. & Kilpatrick, R. (1980). Adrenal gonad relationships. In: General, Comparative and Clinical Endocrinology of the Adrenal Cortex. Vol. 3, 183-264. Eds. I. Chester Jones & I.W. Henderson. Academic Press.
- McKeever, S. (1959). Effects of reproductive activity on the weight of adrenal glands in *Microtus montanus*. Anat. Rec. 135, 1-5.
- Nyholm, N.E.I. & Meurling, P. (1979). Reproduction of the bank vole, *Clethrionomys glareolus* in northern and southern Sweden during several seasons and in different phases of the vole population cycle. Holarctic Ecol. 2, 12-20.
- Pankakoski, E. & Tähkä, K.M. (1982). Relation of adrenal weight to sex, maturity and season in five species of small rodents. Ann. Zool. Fennici 19, 225-232.
- Sealand, J.A. (1967). Reproductive status and adrenal size in the northern red-backed vole in relation to season. Int. J. Biometeorol. 11, 213-220.
- Siuda, S. (1973). The occurrence of X-zone in the adrenal glands of two rodent species. Acta theriol. 18, 471-479.
- Valentine, G.L. & Kirkpatrick, R.O. (1970). Seasonal changes in reproductive and related organs in the pine vole, *Microtus pinetorum*, in southwestern Virginia. J. Mammal. 51, 553-560.

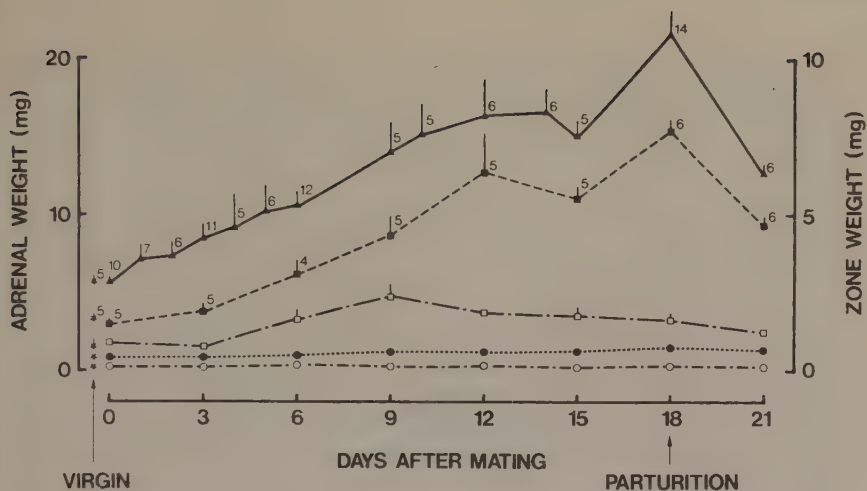


Fig. 1. Paired adrenal weights and weights of cortical zones during pregnancy and early lactation in the bank vole. Values are means $\pm$ S.E. The number of voles for each time-point are shown above each value.

Paired adrenals $\blacktriangle$ — Zona glomerulosa $\bullet$
 Zona fasciculata $\blacksquare$ --- Medulla $\circ$ -----
 Zona reticularis $\square$ -.-.

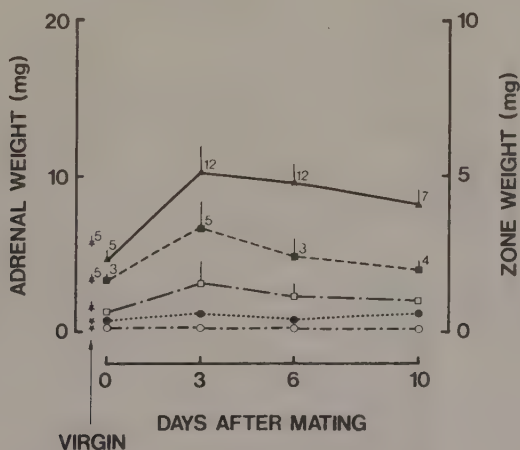


Fig. 2. Paired adrenal weights and weights of cortical zones during pseudopregnancy in the bank vole. Values are means $\pm$ S.E. The number of voles for each time-point are shown above each value. For symbols see Fig. 1.

Table 1. Relative adrenal weight (mean $\pm$ S.E.) during pregnancy in the bank vole

Day of pregnancy	Total body weight		Total body weight - uterine and embryonic weight			n
	Body weight, g	Rel. adrenal weight, mg	Body weight, g	Rel. adrenal weight, mg		
Virgin	17.4 ± 1.6	34.50 ± 5.82	17.4 ± 1.7	34.65 ± 5.86	5	
0	15.8 ± 0.9	36.30 ± 3.76	15.7 ± 0.9	36.50 ± 3.79	10	
3	16.3 ± 0.7	54.07 ± 6.02*	16.2 ± 0.7	54.30 ± 6.00*	11	
6	16.7 ± 0.8	62.60 ± 4.74***	16.6 ± 0.8	63.07 ± 4.78***	10	
9	16.3 ± 0.9	87.80 ± 13.68*	15.9 ± 0.9	90.18 ± 14.25*	5	
12	22.2 ± 1.3	77.62 ± 7.22***	20.3 ± 1.1	85.12 ± 8.16***	5	
15	24.8 ± 0.9	60.08 ± 2.92**	20.0 ± 0.5	73.68 ± 4.27*	4	
18	28.5 ± 1.2	70.37 ± 3.05***	20.0 ± 0.6	105.58 ± 6.66***	14	

Significantly different from day 0 at $P < 0.01^*$ $P < 0.02^{**}$ $P < 0.002^{***}$

Table 2. Adrenal weight (mean $\pm$ S.E.) after the first litter in relation to time after delivery, lactation and post-partum pregnancy

Days after first delivery	Adrenal weight			
	6	n	12	n
<u>Pregnant</u>	Lactating	13.9 $\pm$ 0.5 (5)	14.0 $\pm$ 1.1 (5)	17.7 $\pm$ 0.7 (5)
	Non-lactating	18.0 $\pm$ 3.2 (6)	18.2 $\pm$ 1.2 (6)	21.9 $\pm$ 4.4 (5)
<u>Non-pregnant</u>	Lactating	13.2 $\pm$ 0.7 (5)	12.5 $\pm$ 1.5 (5)	12.9 $\pm$ 1.7 (5)
	Non-lactating	12.8 $\pm$ 1.5 (5)	10.8 $\pm$ 1.1 (4)	10.1 $\pm$ 1.1 (5)

Table 3. Analysis of variance for influence of post-partum conditions on adrenal weight

Source of variation	DF	Mean square	F	Significance
Time after delivery	2	14.414	0.713	N.S.
Lactation	1	27.006	1.336	N.S.
Pregnancy	1	417.841	20.670	P < 0.001

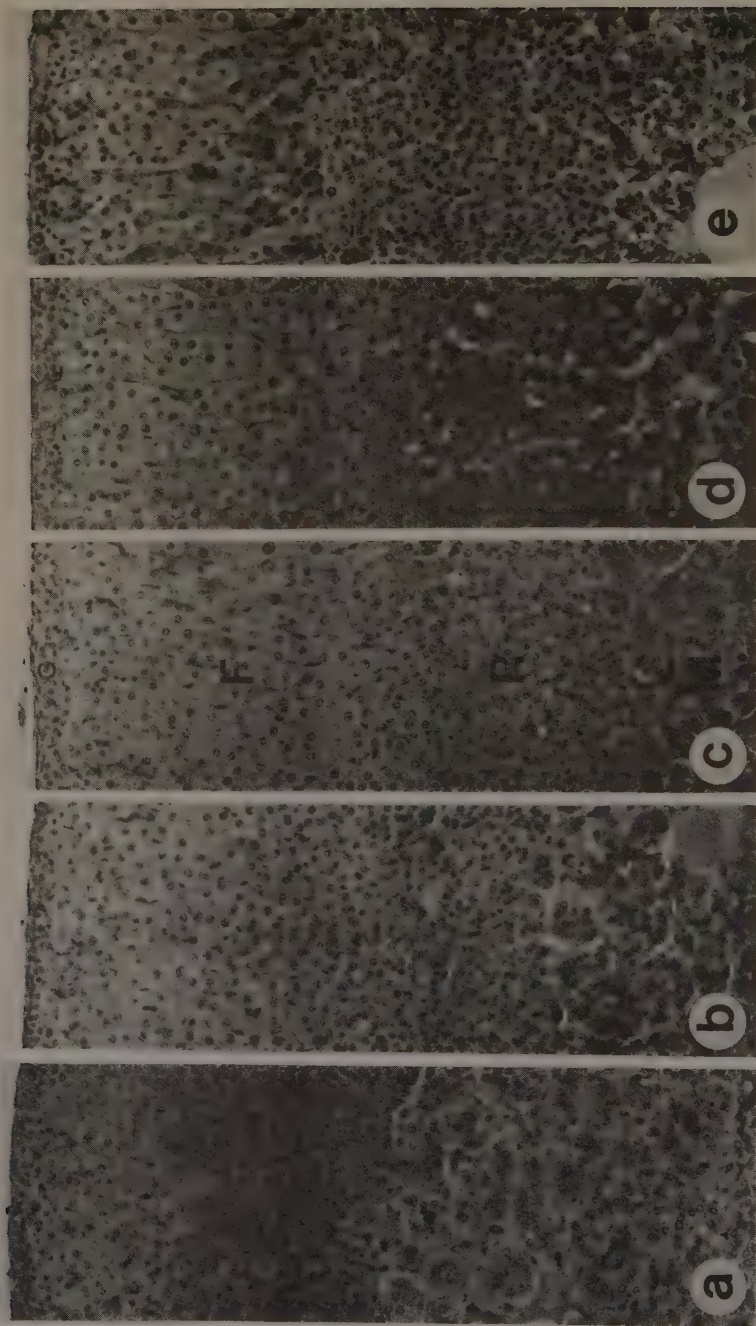


Plate 1. Adrenals of female bank voles showing zona glomerulosa (G), z. fasciculata (F), z. reticularis (R) and medulla (M). a-d, day 0, 6, 12 and 18 of pregnancy and e, day 3 of lactation. H. & E. X160.

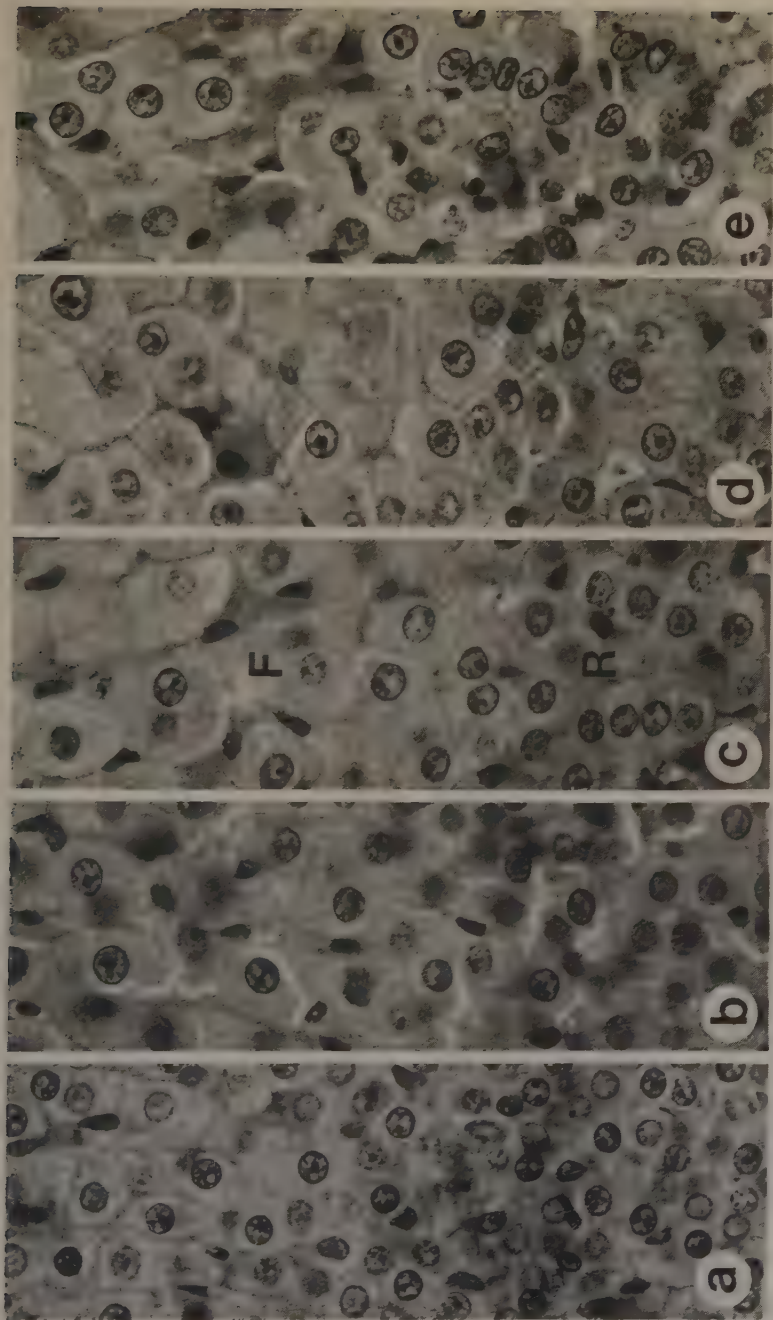


Plate 2. Adrenals of female bank voles showing parts of zona fasciculata (F) and z. reticularis (R). a-d, day 0, 6, 12 and 18 of pregnancy and e, day 3 of lactation. H. & E. X630.

Adrenal growth during pregnancy in the bank vole, *Clethrionomys glareolus*: initiation by mating

TORGNY O. GUSTAFSSON AND C. BERTIL ANDERSSON

Department of Zoology, University of Lund, S-223 62 Lund, Sweden

Received December 4, 1979

GUSTAFSSON, T. O., and C. B. ANDERSSON. 1980. Adrenal growth during pregnancy in the bank vole, *Clethrionomys glareolus*: initiation by mating. *Can. J. Zool.* 58: 1458-1461.

The relative roles of mating and the ovary in the initiating of adrenal growth in pregnant bank voles, *Clethrionomys glareolus*, were studied. In one experiment females were ovariectomized shortly after mating; 4 days later they had significantly heavier adrenals than unmated, ovariectomized controls. In another experiment females were ovariectomized and brought into oestrus by oestradiol-17 β injections. Half of the females were mated and after 4 days their adrenals were significantly larger than those of unmated controls. We conclude that the increase in adrenal weight during pregnancy in bank voles is triggered directly by mating, without involvement of ovarian hormones. The mechanism is suggested to be a neuroendocrine reflex, acting via the hypothalamus and the pituitary. The mechanism may be the same as that involved in the induced ovulation in this species.

GUSTAFSSON, T. O., et C. B. ANDERSSON. 1980. Adrenal growth during pregnancy in the bank vole, *Clethrionomys glareolus*: initiation by mating. *Can. J. Zool.* 58: 1458-1461.

L'influence de la copulation et de l'ovaire sur le déclenchement de la croissance des capsules surrénales chez des campagnols roussâtres femelles enceintes, *Clethrionomys glareolus*, a été étudié. Dans l'une des expériences, des femelles ont subi une ovariectomie quelque temps après l'accouplement; 4 jours plus tard, leurs surrénales étaient plus grosses que celles des femelles témoins opérées mais non accouplées. Au cours d'une autre expérience, les femelles ont subi l'ablation des ovaires et ont été mises en état de rut par des injections de 17- β -oestradiol. La moitié de ces femelles ont été accouplées et au bout de 4 jours, leurs surrénales étaient significativement plus grosses que celles des témoins non accouplés. On peut conclure que l'augmentation de poids des surrénales durant la grossesse, chez ces campagnols, est déclenchée directement par l'accouplement, sans l'intermédiaire des hormones ovariennes. Il semble que ce phénomène soit un réflexe neuro-endocrinien agissant par l'intermédiaire de l'hypothalamus et de l'hypophyse. Le mécanisme de ce phénomène est peut-être le même que celui de l'ovulation provoquée chez cette espèce.

[Traduit par le journal]

Introduction

The adrenal cortex in females of many microtine species is known to increase in size during the reproductive season. This has, for instance, been described in *Microtus agrestis* (Chitty 1961; Chitty and Clarke 1963), *Microtus montanus* (McKeever 1959), *Microtus pennsylvanicus* (Christian and Davis 1966), *Clethrionomys glareolus* (Delost and Delost 1954; Andersson et al. 1978), and *Clethrionomys rutilus* (Sealand 1967). In laboratory studies of *Microtus agrestis* and *Clethrionomys glareolus* the adrenal cortex has been shown to increase greatly in size during pregnancy (Chitty and Clarke 1963; Jorné-Safriel 1968). The growth is confined to the adrenal cortex and the above authors state further that the inner zone of the cortex widens considerably.

In the bank vole the adrenal weight increases three- or four-fold during the 18 days of the first

pregnancy (Jorné-Safriel 1968; C. B. Andersson and T. O. Gustafsson, in preparation).

Various suggestions concerning the hormonal cause for the adrenal growth have been put forward but none of them is based on measurements of hormone levels. Chitty and Clarke (1963) suggest a decrease in androgen levels in the female during pregnancy and (or) the increase in luteinizing hormone (LH) during pregnancy as the cause and Christian and Davis (1966) suggest increased estrogen levels. Jorné-Safriel (1968) showed that in *Clethrionomys glareolus* and *Microtus agrestis*, pseudopregnancy, i.e., mating with vasectomized males, caused adrenal weight increase of the same magnitude as after fertile matings, at least during the first 6 days after mating. Thus it seems clear that the developing embryo plays no part in the triggering of the adrenal growth.

In spite of this there is some relation between

TABLE 1. Body weights and weights of adrenals and uteri (mean $\pm$ SE) 4 days after ovariectomy from 10 littermate pairs of female bank voles, one unmated and one mated shortly before ovariectomy. Ovarian weights recorded at the time of ovariectomy

	<i>n</i>	Body, g	Uteri, g	Adrenals, mg	Ovaries, mg
Mated	10	16.9 $\pm$ 0.37	32.7 $\pm$ 3.99	15.0 $\pm$ 2.05	8.2 $\pm$ 0.53
Unmated	10	18.2 $\pm$ 0.79	19.6 $\pm$ 1.90	8.1 $\pm$ 0.70	6.0 $\pm$ 0.32
<i>p</i> *		N.S.	< 0.025	< 0.01	< 0.025

*Probability according to the Sign test. This test was used as the assumptions required by the *t*-test for paired observations were not always fulfilled.

fertility and adrenal weight increase. In an experiment describing implantation in the bank vole (Andersson and Gustafsson 1979) 68 primiparous females were killed 4 or 6 days after mating. Only half of these were pregnant. The pregnant females had significantly heavier adrenals than the non-pregnant ones.

The experiments described here were designed to show whether or not the adrenal growth is triggered directly by mating, without ovarian involvement.

Material and methods

Experiment 1

The bank voles used were from a colony kept at the Department of Zoology, University of Lund. The animals with which this colony was started were caught in southern Sweden. The animals were kept in plastic cages, measuring 40 cm $\times$ 20 cm $\times$ 15 cm, with wire mesh lids. Wood shavings were used as bedding material and the cages were cleaned twice a week. Standard mouse pellets and water were always available and fresh carrots were supplied twice a week. The light regime was 18 h light – 6 h dark.

From weaning when 16 days old until the start of the experiment at the age of 2–4 months the females used were kept in groups of three to five. At the start of the experiment they were isolated. In this experiment littermate pairs of females were used. One member of each pair was randomly assigned to be mated and subjected to mating tests. The other member of the pair served as a control. In mating tests the male was introduced into the female's home cage in the morning and the pair was kept under observation. If no mating occurred the animals were separated in the evening and the test was repeated the following day. If mating occurred the female was ovariectomized 1–3 h after the initiation of mating, together with its littermate control. The ovariectomy was made through a small dorsal incision, under Nembutal anaesthesia. The ovaries were weighed fresh and fixed in Bouin's fluid. After ovariectomy the females were kept isolated. Four days after the operation the animals were killed and the uteri and adrenals were dissected out, weighed, and fixed. Out of 27 pairs of females, 10 pairs were eventually used. The others were excluded because either the female assigned to be mated did not mate or one member of the pair died during the operation.

Experiment 2

Housing conditions and postweaning procedures were the same as in experiment 1. In experiment 2, however, females from three different colonies of bank voles were used: group A, from the colony originating from southern Sweden described in experiment 1; and groups B and C, from colonies started with

animals from Swedish Lapland, caught in 1976 and 1977, respectively. Eleven females from group A, nine from group B, and eight from group C were used. At the start of the experiment the females were ovariectomized and isolated. The procedure for ovariectomy was the same as in experiment 1 except that Brietal was used as the anaesthetic. After ovariectomy the females were brought into oestrus by three daily injections of oestradiol, 20 μ g/kg body weight (Ovex B, AB Leo, Helsingborg, Sweden), starting on the day after ovariectomy. After the third injection, six randomly selected females in group A, five in group B, and four in group C were paired with males. All females except one in group A and one in group B mated within a few hours. The two which failed to mate were excluded.

Four days after mating the females were killed together with the controls. Their uteri and adrenals were dissected out, weighed fresh, and fixed.

Results and discussion

Experiment 1

Ovarian weights at ovariectomy, body weights, and the weights of adrenals and uteri measured on completion of the experiment are given in Table 1. The mated females had significantly heavier adrenals than the unmated animals. The uteri were also significantly larger.

These results indicate that the adrenal glands grow in early pregnancy as a direct consequence of mating, without ovarian involvement. However, in this experiment the results can only be taken as indicative, as three main drawbacks can be identified. (1) The females were strongly selected as some pairs were excluded when the female assigned to be mated failed to mate. (2) The controls and the mated females were not in the same physiological state at the start of the experiment. All the mated females were in oestrus whereas only a few of the unmated ones were. This is reflected in the significant difference between the mean ovarian weights (Table 1). Moreover, as some of the mated females had been paired with males for several consecutive days before mating, social effects cannot be excluded. (3) The ovaries were present for a few hours after mating and a rapid change in ovarian hormone release could be involved. To overcome the above difficulties experiment 2 was performed.

TABLE 2. Ovarian weights (at the time of ovariectomy) (mean $\pm$ SE) from the bank voles in experiment 2

	<i>n</i>	Ovaries, mg
Group A		
Mated*	4	4.5 $\pm$ 0.32
Unmated	5	5.2 $\pm$ 0.29
Group B		
Mated*	4	3.8 $\pm$ 0.55
Unmated	4	3.0 $\pm$ 0.52
Group C		
Mated*	4	4.7 $\pm$ 0.21
Unmated	4	6.3 $\pm$ 1.63

NOTE: No significant difference between mated and unmated females was detected. (Two way ANOVA, $F = 0.69$.)

*Mating took place 3 days after ovariectomy.

Experiment 2

Ovarian weights at ovariectomy and adrenal, uterine, and body weights measured on completion of the experiment are given in Tables 2 and 3.

There were no differences in ovarian weight between mated and unmated females at the start of the experiment. At the end of the experiment, however, the mated females in all the groups had heavier adrenals than their unmated counterparts. This difference was highly significant. No significant differences between treatments in uterine or body weights could be seen, although mated females in all groups had slightly larger uteri.

The reason for the enlarged uteri in the mated animals from experiment 1 and a tendency in the same direction in experiment 2 is unclear. It may simply be a consequence of the filling of the uteri with sperm 4 days earlier. It may also be a result of selection or the oestrus state, as discussed above,

or a consequence of changed hormone levels, either of pituitary or adrenal origin.

From this experiment it is concluded that mating causes adrenal hypertrophy directly, without involvement of the ovary. The mechanism for this is probably a neuroendocrine reflex triggered by mating. Bank voles are induced ovulators (Clarke et al. 1970), as are most microtines (Hasler 1975). The mechanism involved in adrenal growth may well be related to the ovulation mechanism.

Hormonal events underlying ovulation and corpus luteum function in *Microtus agrestis* have been studied by Milligan and MacKinnon (1976). After the first intromission the level of LH rises and the female ovulates. However, for the activation of the corpora lutea it is necessary that the first intromission is followed by further stimulation of the vagina. This stimulation is associated with a rise in the prolactin levels and prolonged elevated LH levels. A similar mechanism has been reported for *Microtus montanus* (McM Kenney and Dewsbury 1977). These hormonal events at mating have not been related to the adrenal.

To our knowledge, the levels of pituitary hormones other than LH and prolactin have not been studied in relation to mating and pregnancy in any microtine rodents.

Acknowledgements

This work was supported by grants from the Swedish Natural Science Research Council (to Dr. P. Meurling) and from the Royal Physiographic Society of Lund. We wish to thank AB Leo, Helsingborg, for providing the Ovex B. Thanks are due to Dr. P. Meurling and Dr. N. E. I. Nyholm for criticizing the manuscript. We are also indebted to Mrs. M. Fisher for linguistic assistance and to Mrs. I. Mortensen for animal maintenance.

TABLE 3. Body weights and weights of adrenals and uteri (mean $\pm$ SE) from the female bank voles in experiment 2

	<i>n</i>	Body weight, g	Uterine weight, mg	Adrenal weight, mg
Group A				
Mated	5	16.4 $\pm$ 0.31	47.7 $\pm$ 3.84	8.84 $\pm$ 0.910
Unmated	5	17.1 $\pm$ 0.49	39.2 $\pm$ 1.06	6.60 $\pm$ 0.410
Group B				
Mated	4	16.6 $\pm$ 0.28	25.4 $\pm$ 4.80	8.33 $\pm$ 1.269
Unmated	4	17.2 $\pm$ 0.91	23.8 $\pm$ 3.81	5.70 $\pm$ 0.474
Group C				
Mated	4	17.6 $\pm$ 0.97	41.8 $\pm$ 2.80	9.90 $\pm$ 0.895
Unmated	4	18.2 $\pm$ 1.06	38.3 $\pm$ 6.34	7.00 $\pm$ 0.896

NOTE: Mated females have larger adrenals than unmated ones (two-way ANOVA, F treatment = 13.93, $df = 1, 20$, $p < 0.001$). No treatment effect on body weight or uterine weight could be seen (F values of 1.06 and 2.248, respectively).

- ANDERSSON, B., T. GUSTAFSSON, P. MEURLING, and E. NYHOLM. 1978. The role of the adrenal cortex in reproduction in the female bank vole, *Clethrionomys glareolus*. In *Comparative endocrinology*. Edited by P. J. Gaillard and H. H. Boer. Elsevier/North Holland, Amsterdam, p. 83.
- ANDERSSON, C. B., and T. O. GUSTAFSSON. 1979. Delayed implantation in lactating female bank voles (*Clethrionomys glareolus*). *J. Reprod. Fertil.* 57: 349-352.
- CHITTY, H. 1961. Variations in the weight of the adrenal glands of the field vole, *Microtus agrestis*. *J. Endocrinol.* 22: 387-393.
- CHITTY, H., and J. R. CLARKE. 1963. The growth of the adrenal glands of laboratory and field voles, and changes in it during pregnancy. *Can. J. Zool.* 41: 1025-1034.
- CHRISTIAN, J. J., and D. E. DAVIS. 1966. Adrenal glands in female voles (*Microtus pennsylvanicus*) as related to reproduction and population size. *J. Mammal.* 47: 1-18.
- CLARKE, J. R., F. V. CLULOW, and F. GREIG. 1970. Ovulation in the bank vole, *Clethrionomys glareolus*. *J. Reprod. Fertil.* 25: 531.
- DELOST, P., and H. DELOST. 1954. Existence d'une zone X surrénalienne chez le Campagnol roussâtre (*Clethrionomys glareolus* S.). *C. R. Soc. Biol.* 148: 1788-1791.
- HASLER, J. F. 1975. A review of reproduction and sexual maturation in the microtine rodents. *Biologist*, 57: 52-86.
- JORNÉ-SAFRIEL, O. 1968. Some factors affecting the adrenal juxtamedullary zone in the vole, *Microtus agrestis*, and bank vole, *Clethrionomys glareolus*. Ph. D. thesis. Oxford University.
- McKEEVER, S. 1959. Effects of reproductive activity on the weight of adrenal glands in *Microtus montanus*. *Anat. Rec.* 135: 1-5.
- McKENNEY, A., and D. A. DEWSBURY. 1977. Effect of limited mating on the corpora lutea in montane voles, *Microtus montanus*. *J. Reprod. Fertil.* 49: 363-364.
- MILLIGAN, S. R., and P. C. B. MAC KINNON. 1976. Correlation of plasma LH and prolactin levels with the fate of the corpus luteum in the vole, *Microtus agrestis*. *J. Reprod. Fertil.* 47: 111-113.
- SEALANDER, J. A. 1967. Reproductive status and adrenal size in the northern red-backed vole in relation to season. *Int. J. Biometeorol.* 11: 213-220.

Relation between fertility and adrenal growth after mating in the bank vole, *Clethrionomys glareolus*

BERTIL ANDERSSON¹ AND TORGNY GUSTAFSSON

Department of Zoology, University of Lund, S-223 62 Lund, Sweden

Received June 10, 1980

ANDERSSON, B., and T. GUSTAFSSON. 1981. Relation between fertility and adrenal growth after mating in the bank vole, *Clethrionomys glareolus*. Can. J. Zool. 59: 329-331.

The relationship between adrenal status and fertility in primiparous female bank voles, *Clethrionomys glareolus*, was examined. Out of 68 primiparous females, killed 4.5 or 6 days postcoitum, only 36 were pregnant. The pregnant ones had significantly larger adrenals than the nonpregnant ones. The nonpregnant females also had smaller ovaries, but the presence of corpora lutea proved that ovulation had occurred. Similarly, only 5 out of 11 females, mated with vasectomized males, were pseudopregnant with large corpora lutea, 6 days postcoitum. The remaining ones had ovaries similar to those in the other nonpregnant females. The pseudopregnant females had significantly larger adrenals than the nonpseudopregnant. This relationship between fertility and adrenal growth is unexpected, as in previous experiments an increase in adrenal weight has been shown to take place in direct response to mating, in the absence of ovaries and embryos.

ANDERSSON, B., et T. GUSTAFSSON. 1981. Relation between fertility and adrenal growth after mating in the bank vole, *Clethrionomys glareolus*. Can. J. Zool. 59: 329-331.

La relation entre l'état des surrénales et la fertilité a été étudiée chez des femelles primipares du campagnol roussâtre, *Clethrionomys glareolus*. De 68 femelles primipares sacrifiées 4, 5 ou 6 jours après l'accouplement, seulement 36 étaient fécondées. Les femelles gravides avaient les surrénales significativement plus grosses que les femelles qui ne l'étaient pas. Les femelles qui n'étaient pas gravides avaient aussi de plus petits ovaires, mais la présence des corps jaunes démontrait qu'il y avait eu ovulation. De 11 femelles accouplées à des mâles qui avaient subi la vasectomie, seulement 5 avaient les symptômes d'une pseudo-grossesse et des corps jaunes de grande taille, 6 jours après l'accouplement. Chez les autres, les ovaires étaient semblables à ceux des autres femelles non gravides. Les femelles pseudo-gravides avaient les surrénales significativement plus grosses que les femelles non pseudo-gravides. Cette relation entre la fertilité et la croissance des surrénales est inattendue, car des expériences antérieures ont démontré qu'il se produit une augmentation du poids des surrénales à la suite de l'accouplement, même en l'absence d'ovaires ou d'embryons.

[Traduit par le journal]

Introduction

In many female microtines, the adrenal cortex increases in size during the breeding season. An increase in adrenal weight in sexually active females has been described in, for instance, *Microtus agrestis* (Chitty 1961; Chitty and Clarke 1963), *M. montanus* (McKeever 1959), *M. pennsylvanicus* (Christian and Davis 1966), *Clethrionomys glareolus* (Delost and Delost 1954; Andersson *et al.* 1978), and in *C. rutilus* (Sealander 1967).

The increase in adrenal weight in *M. agrestis* and *C. glareolus* has been shown to take place during the first pregnancy (Jorné-Safriel 1968; Chitty and Clarke 1963). In the bank vole, the adrenal weight increases approximately three- to four-fold during the 18 days of the first pregnancy (Jorné-Safriel 1968; Andersson *et al.* 1978). According to Chitty and Clarke (1963) and Jorné-Safriel (1968) only the cortex increases in size. Particularly the inner zone, called X-zone or juxtamedullary zone, is affected.

Pseudopregnancy may cause adrenal growth in *C. glareolus* and *M. agrestis* (Jorné-Safriel 1968), and

ovariectomized, oestradiol-treated females show adrenal growth after mating (Gustafsson and Andersson 1980a). Thus, adrenal growth in response to mating can take place in the absence of embryos and ovaries.

The relationship between adrenal status and fertility was studied in the present experiment.

Material and methods

Bank voles, *Clethrionomys glareolus*, from a colony founded on animals caught in southern Sweden and kept in our laboratory were used (see Gustafsson *et al.* 1980). The animals were kept in plastic cages, measuring 40 cm × 20 cm × 15 cm, with wire mesh lids. Wood shavings were used as bedding and the cages were cleaned twice a week. Standard laboratory mouse pellets (AB Harald Fors & Co., Vadstena, Sweden) and water were always available and fresh carrots were supplied twice a week. The light regime was 18 h light (L): 6 h dark (D).

From weaning, when 16 days old, the females were kept in groups of 4-5 and at the start of the experiment, at 2-5 months of age, they were placed in separate cages. After 1-2 days an adult male was introduced to each female in the morning and the pair was checked every 1-2 h to see if mating had occurred. Two to four hours after an observed mating, or after the finding of a vaginal plug, the male was removed. If mating did not

¹Author to whom reprint requests should be sent.

TABLE 1. Mean weights ($\pm$ SE) of ovaries and adrenals 4.5 and 6 days after mating in female bank voles, in relation to outcome of mating

Condition		Autopsy days pc	n	Ovarian weight, mg	Adrenal weight, mg
Intact male	Nonpregnant	4.5	19	13.6 $\pm$ 0.90	8.5 $\pm$ 0.60 ^a
	Pregnant	4.5	24	22.3 $\pm$ 0.83	15.0 $\pm$ 1.26 ^{a,d}
Intact male	Nonpregnant	6	13	12.1 $\pm$ 0.99	11.9 $\pm$ 3.24 ^b
	Pregnant	6	12	21.4 $\pm$ 1.68	20.1 $\pm$ 2.94 ^{b,d}
Vasectomized male	Nonpseudo-pregnant	6	6	7.2 $\pm$ 1.04	5.8 $\pm$ 0.78 ^c
	Pseudopregnant	6	5	16.1 $\pm$ 0.49	11.4 $\pm$ 1.58 ^c

NOTE: Means denoted by the same letter (a-d) are significantly different, $P < 0.01$, Mann-Whitney U-test.

occur during the day, the male was removed and reintroduced the following day.

Sixty-eight females mated with intact males and 11 females mated with vasectomized males were used. Those mated with intact males were also used in a study of delayed implantation (Andersson and Gustafsson 1979). They were killed 4.5 or 6 days postcoitum (pc) and those mated with vasectomized males were killed 6 days pc. Reproductive organs and adrenals were dissected out, weighed fresh, and fixed in a mixture of 96% ethanol, neutral formalin, and glacial acetic acid (proportions 15:4:1). The uteri of females killed 4.5 days pc were embedded in paraffin wax, serially sectioned (7 μ m), stained with Ehrlich haematoxylin-eosin, and examined for the presence of blastocysts. In the day 6 pregnant females visible implantation sites were present.

Mann-Whitney's U-test was used instead of Student's *t*-test, as the distribution of adrenal weights was markedly non-normal in most cases.

Results

Forty-three primiparous females were killed 4.5 days pc. Only 24 of these were pregnant, with blastocysts about to implant; their ovaries contained large (functional) corpora lutea. In the remaining 19 females no blastocysts were found; these nonpregnant animals had small ovaries and small (short-lived) corpora lutea. Similarly, only 12 out of 25 females killed 6 days pc were pregnant. Adrenal weights were significantly higher in pregnant than in nonpregnant females, both 4.5 and 6 days pc (Table 1). Furthermore, the adrenals of 6 days pregnant females were heavier than those of 4.5 days pregnant ones (Table 1).

Mating with a vasectomized male led to pseudopregnancy with large (functional) corpora lutea in five females, whereas the remaining six had small ovaries and small (short-lived) corpora lutea, similar to those of the nonpregnant females mentioned above. The pseudopregnant females had significantly larger adrenals than the nonpseudopregnant ones (Table 1).

Discussion

The mechanism which regulates adrenal growth after

mating has only been investigated in a few experiments. In *C. glareolus* and *M. agrestis*, the adrenal weight increases steadily throughout pregnancy (Jorné-Safriel 1968; Andersson and Gustafsson 1980). Therefore, the difference in adrenal weight found between 4.5 and 6 days pregnant females was expected. In both species, pseudopregnancy causes adrenal weight increase of a similar magnitude to that seen during pregnancy (Jorné-Safriel 1968; Andersson and Gustafsson 1980). In ovariectomized, oestradiol-treated female *C. glareolus*, mating also results in adrenal growth (Gustafsson and Andersson 1980a). These experiments show that neither ovaries nor embryos are necessary for the triggering of adrenal growth, and suggest that mating acts directly on the hypothalamus and (or) the pituitary. Thus, the relationship between fertility and adrenal growth demonstrated here is somewhat unexpected.

The low fertility in primiparous females in this experiment is consistent with findings in field populations of *C. glareolus* (Coulters and Rowlands 1969). Similar findings have been reported for *M. agrestis* (Milligan and MacKinnon 1976).

The presence of small corpora lutea in nonpregnant and nonpseudopregnant females shows that the low fertility is not caused by a lack of ovulation. Further, the presence of large corpora lutea in some of the females mated with vasectomized males suggests that lack of fertilization is not the reason for the low fertility.

Clethrionomys glareolus and *M. agrestis* are induced ovulators (Clarke *et al.* 1970; Breed and Clarke 1970). The mechanism for ovulation and activation of the corpora lutea in *M. agrestis* has been described by Milligan (1975). Ovulation is induced by the first few intromissions, but a further mechanical stimulation of the vagina is necessary for the corpora lutea to become functional. A similar mechanism is found in *C. glareolus* (Gustafsson and Andersson 1980b). The low fertility in primiparous females could be explained if the stimulation threshold for activation of the corpora lutea is not always reached at mating.

If ovaries and adrenals are activated by the same mechanisms, the relationship between outcome of mating and adrenal growth is explained.

Acknowledgements

This work was supported by grants from the Swedish Natural Science Research Council and from The Royal Physiographic Society of Lund. The authors are indebted to Dr. C. E. Adams, Cambridge, for valuable comments, to Mrs. M. Fisher, Oxford, for assistance with the English, and to Mrs. I. Mortensen for animal care.

ANDERSSON, C. B., and T. O. GUSTAFSSON. 1979. Delayed implantation in lactating bank voles, *Clethrionomys glareolus*. *J. Reprod. Fertil.* 57: 349–352.

———. 1980. The adrenal cortex during and after pregnancy in the bank vole, *Clethrionomys glareolus*. *Gen. Comp. Endocrinol.* 40: 343.

ANDERSSON, B., T. GUSTAFSSON, P. MEURLING, and E. NYHOLM. 1978. The role of the adrenal cortex in reproduction in the female bank vole, *Clethrionomys glareolus*. In *Comparative endocrinology*. Edited by P. J. Gaillard and H. H. Boer. Elsevier/North-Holland, Amsterdam and New York, p. 83.

BREED, W. G., and J. R. CLARKE. 1970. Ovulation and associated histological changes in the ovary following coitus in the vole (*Microtus agrestis*). *J. Reprod. Fertil.* 22: 173–175.

CHITTY, H. 1961. Variations in the weight of the adrenal glands of the field vole, *Microtus agrestis*. *J. Endocrinol.* 22: 387–393.

CHITTY, H., and J. R. CLARKE. 1963. The growth of the adrenal glands of laboratory and field voles and changes in it during pregnancy. *Can. J. Zool.* 41: 1025–1034.

CHRISTIAN, J. J., and D. E. DAVIS. 1966. Adrenal glands in female voles (*Microtus pennsylvanicus*) is related to reproduction and population size. *J. Mammal.* 47: 1–18.

CLARKE, J. R., F. V. CLULOW, and F. GREIG. 1970. Ovulation in the bank vole, *Clethrionomys glareolus*. *J. Reprod. Fertil.* 25: 531.

COUTTS, R. R., and I. W. ROWLANDS. 1969. The reproductive cycle of the skomer vole, *Clethrionomys glareolus skomerensis*. *J. Zool. (London)*, 158: 1–25.

DELOST, P., and H. DELOST. 1954. Existence d'une zone X surrénalienne chez le Campagnol roussatre (*Clethrionomys glareolus* S.). *C.R. Soc. Biol.* 148: 1788–1791.

GUSTAFSSON, T. O., and C. B. ANDERSSON. 1980a. Adrenal growth during pregnancy in the bank vole, *Clethrionomys glareolus*: initiation by mating. *Can. J. Zool.* 58: 1458–1461.

———. 1980b. Effect of limited and complete mating on ovaries and adrenals of bank voles, *Clethrionomys glareolus*. In *Program and Abstracts Annual Conference 1980. Society for the Study of Fertility*. Abstr. 74.

GUSTAFSSON, T. O., C. B. ANDERSSON, and L. WESTLIN. 1980. Reproduction in a laboratory colony of bank vole, *Clethrionomys glareolus*. *Can. J. Zool.* 58: 1016–1021.

JORNÉ-SAFRIEL, O. 1968. Some factors affecting the adrenal juxtamedullary zone in the vole, *Microtus agrestis* and bank vole, *Clethrionomys glareolus*. Ph.D. thesis, Oxford University, Oxford.

McKEEVER, S. 1959. Effects of reproductive activity on the weight of adrenal glands in *Microtus montanus*. *Anat. Rec.* 135: 1–5.

MILLIGAN, S. R. 1975. Mating, ovulation and corpus luteum-function in the vole, *Microtus agrestis*. *J. Reprod. Fertil.* 42: 35–44.

MILLIGAN, S. R., and P. C. B. MACKINNON. 1976. Correlation of plasma LH and prolactin levels with the fate of the corpus luteum in the vole, *Microtus agrestis*. *J. Reprod. Fertil.* 47: 111–113.

SEALANDER, J. A. 1967. Reproductive status and adrenal size in the northern red-backed vole in relation to season. *Int. J. Biometeorol.* 11: 213–220.

Effect of limited and complete mating on ovaries and adrenals in bank voles, *Clethrionomys glareolus*

C. B. Andersson and T. O. Gustafsson

Department of Zoology, University of Lund, S-223 62 Lund, Sweden

Summary. Complete mating of voles induced ovulation and large and healthy CL were formed. By 4 days after mating adrenal weight was twice that in unmated animals. Limited amounts of mating (i.e. 1–3 intromissions) induced ovulation, but the resulting CL were small and short-lived and no adrenal hypertrophy was seen. If limited mating was followed by mechanical genital stimulation, large CL and adrenal hypertrophy were induced. Mechanical genital stimulation alone induced ovulation (mostly small CL) in some females, but not adrenal hypertrophy. It is concluded that adrenal hypertrophy after mating in the bank vole is controlled by a neuroendocrine reflex mechanism separate from that controlling ovulation, but related to that controlling luteal function.

Introduction

Ovulation in all microtine rodents hitherto studied is induced by mating (see review by Hasler, 1975). However, studies of *Microtus montanus* (Davis, Gray, Zerylnick & Dewsbury, 1974), *M. ochrogaster* (Gray, Zerylnick, Davis & Dewsbury, 1974), *M. agrestis* (Milligan, 1975a), *M. pennsylvanicus* (Gray, Kenney & Dewsbury, 1977) and *M. californicus* (Kenney, Hartung & Dewsbury, 1979) show that the amount of mating stimulation necessary for ovulation and pregnancy varies with the species studied. In each species there seems to exist an association between male copulatory pattern and the stimulation requirements by the female for initiation of pregnancy (Gray & Dewsbury, 1973, 1975). An unrestricted mating induces both ovulation and luteal function; if mating is limited it can still induce ovulation in *M. agrestis* and *M. montanus*, but the resulting CL are short-lived (Milligan, 1975a; Kenney & Dewsbury, 1977). In the field vole, *M. agrestis*, at least, the development of luteal function is controlled by a neuroendocrine reflex mechanism which is distinct from that controlling ovulation. Both mechanisms are activated by mating, but by different amounts of stimulation (Milligan, 1975a). *Microtus ochrogaster* and *M. californicus* differ in that even limited mating results in ovulation and a functional luteal phase (Gray *et al.*, 1974; Kenney *et al.*, 1979).

The copulatory patterns within the genus *Microtus* show many similarities. They are characterized by the absence of a copulatory lock, multiple ejaculations and prolonged intromissions accompanied by pelvic thrusting. In *M. montanus*, *M. ochrogaster* and *M. agrestis* ejaculation is preceded by multiple intromissions (Dewsbury, 1973; Gray & Dewsbury, 1973, 1975; Milligan, 1975b; Kenney *et al.*, 1979).

A different mating pattern is found in *Clethrionomys glareolus*, another microtine. Bank voles show a multiple intromission/multiple ejaculation mating pattern, but intromissions are very brief (1–2 sec) without pelvic thrusting (Milligan, 1979). The relationship between this mating pattern and the induction of ovulation and luteal function has not been investigated.

In bank voles and field voles the adrenals increase in weight during pregnancy and

pseudopregnancy (Jorné-Safriel, 1968). The change in bank voles is a 3–4-fold increase at the end of pregnancy (Andersson & Gustafsson, 1980) and it has been shown to be induced directly by mating (Gustafsson & Andersson, 1980).

The effect of different amounts of mating on the adrenals and ovaries of bank voles was studied in the present paper.

Materials and Methods

Laboratory bred bank voles, *Clethrionomys glareolus*, were used (see Gustafsson, Andersson & Westlin, 1980). From weaning at 16 days until the beginning of the experiment, the females were kept in groups of 4–5. At the start of the experiment the animals were aged 2–4 months and were caged singly for at least 2 days and then randomly divided into 5 treatment groups: Group 1, unmated; Group 2, limited mating (1–3 intromissions); Group 3, complete mating; Group 4, mechanical genital stimulation; Group 5, limited mating followed by mechanical genital stimulation. An adult male was introduced to each female in Groups 2, 3 and 5 in the morning and the pair was either kept under constant observation until mating (Groups 2 and 5) or checked for mating every 1–2 h (Group 3). The male was removed after 1–3 intromissions (Groups 2 and 5), as judged by mating behaviour (Christiansen & Døving, 1976), or left with the male for 2–4 h after initiation of mating (Group 3). If mating did not occur during the day, the male was removed and re-introduced the following day. Females that had not mated after 5 mating trials, were discarded.

In an attempt to mimic a complete mating, for some females limited mating was followed by mechanical genital stimulation (Group 5) with a plastic rod (2 × 20 mm, vibrating at 50 Hz) attached to a modified electric razor. The rod was placed in the vagina for 2 sec at 15-sec intervals during a 1-min period. Ten such 1-min stimulation periods were given at 10-min intervals. The stimulation regimen was based on observations of the mating behaviour of bank voles (Christiansen & Døving, 1976; Milligan, 1979).

One group of females was subjected to mechanical genital stimulation only to check whether it could induce ovulation and adrenal hypertrophy.

Animals in Groups 2–5 were killed 4 days after treatment, while the unmated controls were killed 7–14 days after grouping. At autopsy the ovaries, adrenals and uteri were weighed fresh and the ovaries were examined for CL. The ovaries were fixed in Bouin's fluid, embedded in paraffin wax, serially sectioned at 7 µm and stained with Ehrlich's haematoxylin and eosin. The diameters of CL were measured as the mean of two measurements at right angles through the largest section of each CL, using an eye-piece micrometer.

Results

The proportion of females ovulating and the appearance of CL at autopsy after different amounts of mating is shown in Table 1. The CL were large and vascular, as characteristic of pregnancy and pseudopregnancy (functional), or small and pale (short-lived). All the CL classified as functional at autopsy proved histologically healthy, while those found to be regressing were identifiable with the ones classified as short-lived.

Only one of the unmated females had ovulated and showed regressing CL at autopsy. In most females limited mating was sufficient to induce ovulation, but the resulting CL were short-lived. All females ovulated after a complete mating and most of them had functional CL. Almost half of the females subjected to mechanical genital stimulation only had ovulated, but only one of them showed functional CL. Limited mating followed by mechanical genital stimulation resulted in ovulation and functional CL in a majority of the animals.

*Limited and complete mating in bank voles***Table 1.** Body weight ($\pm$ s.e.m.) and the effect of different amounts of mating on ovulation and appearance of corpora lutea in female bank voles

Group	Treatment	No. of voles	No. ovulating		No. not ovulating	Body wt (g)
			Short-lived CL	Healthy CL		
1	Unmated	21	1	0	20	17.4 $\pm$ 0.5
2	Limited mating	18	15	0	3	17.4 $\pm$ 0.6
3	Complete mating	16	2	14	0	17.2 $\pm$ 0.6
4	Mechanical stimulation	16	6	1	9	16.8 $\pm$ 0.7
5	Limited mating + mechanical stimulation	20	6	12	2	17.9 $\pm$ 0.4

Table 2. Mean $\pm$ s.e.m. weights of ovaries and adrenals, and diameter of CL from female bank voles after different amounts of mating (see Table 1, no. of CL measured are given in parenthesis)

	Group 1 (unmated)	Group 2 (limited mating)	Group 3 (complete mating)	Group 4 (mechanical stimulation)	Group 5 (limited mating + mechanical stimulation)
Ovarian wt (mg)					
♀♀ not ovulating	5.4 $\pm$ 0.3	8.3 $\pm$ 1.2	—	6.2 $\pm$ 0.7	8.7 $\pm$ 0.1
♀♀ with short-lived CL	13.2	12.4 $\pm$ 0.5†	11.1 $\pm$ 1.2†	10.3 $\pm$ 1.9†	12.8 $\pm$ 0.8†
♀♀ with functional CL	—	—	20.1 $\pm$ 1.0*	24.1	21.1 $\pm$ 1.5*
CL diameter (mm)					
♀♀ with short-lived CL	1.21 (2)	1.13 $\pm$ 0.02 (42)	1.00 $\pm$ 0.05 (6)	1.04 $\pm$ 0.03 (14)	1.05 $\pm$ 0.06 (18)
♀♀ with healthy CL	—	—	1.45 $\pm$ 0.03 (37)*	1.6 (1)	1.41 $\pm$ 0.04 (40)*
Adrenal wt (mg)					
♀♀ not ovulating	5.2 $\pm$ 0.4	7.2 $\pm$ 1.6	—	5.5 $\pm$ 0.6	5.5 $\pm$ 1.2
♀♀ with short-lived CL	7.0	6.9 $\pm$ 0.5	6.6 $\pm$ 1.6	7.0 $\pm$ 1.5	5.8 $\pm$ 0.7
♀♀ with healthy CL	—	—	11.3 $\pm$ 1.3*	11.5	15.6 $\pm$ 1.6*

* Significantly different from females with short-lived CL, $P < 0.05$ (LSD method of multiple comparisons).

† Significantly different from unmated females, $P < 0.05$ (LSD method of multiple comparisons).

There were no differences in body weight amongst the 5 groups (Table 1), but ovarian weights did vary with the type of CL formed (Table 2). The ovaries with functional CL were heaviest but there was also a significant difference in ovarian weight between females with short-lived CL and unmated females. The functional CL were significantly larger than the short-lived CL (Table 2).

A marked increase in adrenal weight occurred in females with functional CL, while adrenal weight in females with short-lived CL did not differ from that in unmated animals (Table 2).

Discussion

The present results suggest that, as in field voles (Milligan, 1975a), mating in bank voles activates two neuroendocrine reflex mechanisms which separately induce ovulation and the development of luteal function. Both mechanisms are normally activated by an unrestricted mating, while a limited mating stimulates ovulation but no functional luteal phase follows. However, the stimulation threshold for ovulation seems to be lower in bank voles than in other microtines. Ovulation is readily induced by 1–3 intromissions of 1–2 sec each, while in field

voles, for instance, it requires an intromission of about 20 sec to induce ovulation (Milligan, 1975a). Therefore, in bank voles, as in several other microtine species (Gray *et al.*, 1977), there appears to be a mutual adaptation between the male mating pattern and the female stimulation requirements for the induction of ovulation and luteal function.

Mechanical genital stimulation can mimic part of the mating act in field voles (Milligan, 1975a), causing CL to become functional after an ovulatory stimulus (LH-RH or intromission). Complete mating in bank voles can also be mimicked by a few intromissions followed by mechanical genital stimulation. However, in addition to this and in contrast to previous studies of *M. californicus*, *C. glareolus* and *M. agrestis* (Greenwald, 1956; Clarke & Clulow, 1973; Milligan, 1975a), the present results show that total artificial genital stimulation can induce ovulation in some bank voles. This is not unexpected when very limited mating, i.e. a few 1–2 sec intromissions, is effective in inducing ovulation. The fact that ovulation was not induced in all bank voles by mechanical stimulation is probably due to failure to mimic some factor of male stimulation during mating. It could be a lack of additional mechanical stimulation (e.g. penile spines, Zarrow & Clark, 1968) or of other male stimuli. The importance of ovulatory stimuli other than mating has been discussed by Milligan (1974, 1975a) who showed that in field voles mounting without intromission or the mere presence of males could induce ovulation. Stimuli other than mating can induce ovulation in bank voles (Clarke & Hellwing, 1977). Indeed, one of the isolated females in Group 1 of this study ovulated. The experimental females were kept in the same animal room as the breeding colony and therefore exposed to a variety of stimuli.

As expected, adrenal weight was roughly doubled by 4 days after mating compared to that of unmated animals (Andersson & Gustafsson, 1980). This effect on the adrenals, induced by unrestricted mating (Gustafsson & Andersson, 1980), was mimicked by limited mating followed by mechanical genital stimulation, but not by limited mating alone. These results show that, like luteal function, induction of adrenal hypertrophy requires stronger stimulation than does induction of ovulation. Irrespective of the ovulatory stimulation given, ovulation in field voles is preceded by a marked elevation of the plasma LH concentrations, but is followed by a functional luteal phase only if prolactin levels are also elevated, as they are after unrestricted mating (Milligan, 1976). However, in non-pregnant, lactating bank voles in which the prolactin levels are assumed to be fairly high, adrenal hypertrophy is not sustained (Andersson & Gustafsson, 1980). We conclude that in bank voles a neuroendocrine reflex mechanism, separate from that controlling ovulation, but probably related to that controlling luteal function, must be stimulated to induce adrenal hypertrophy after mating. The nature of this mechanism is being investigated.

This work was supported by grants from the Swedish Natural Science Research Council and the Royal Physiographic Society of Lund. We are indebted to Miss M. Niklasson for expert technical assistance.

References

- Andersson, B. & Gustafsson, T. (1980) The adrenal cortex during and after pregnancy in the bank vole, *Clethrionomys glareolus*. *Gen. comp. Endocr.* **40**, 343, Abstr.
- Christiansen, E. & Døving, K.B. (1976) Observations of the mating behavior of the bank vole, *Clethrionomys glareolus*. *Behav. Biol.* **17**, 263–266.
- Clarke, J.R. & Clulow, F.W. (1973) Effect of successive matings upon bank vole, *Clethrionomys glareolus*, and vole, *Microtus agrestis*, ovaries. In *The Development and Maturation of the Ovary and its Functions*, pp. 160–170. Ed. H. Peters. Excerpta Medica (I.C.S. No. 267), Amsterdam.
- Clarke, J.R. & Hellwing, S. (1977) Remote control by males of ovulation in bank voles, *Clethrionomys glareolus*. *J. Reprod. Fert.* **50**, 155–158.
- Davis, H.N., Gray, G.D., Zerylnick, M. & Dewsbury, D.A. (1974) Ovulation and implantation in montane voles, *Microtus montanus*, as a function of varying amounts of copulatory stimulation. *Horm. Behav.* **5**, 383–388.
- Dewsbury, D.A. (1973) Copulatory behavior of montane voles (*Microtus montanus*). *Behaviour* **44**, 186–202.
- Gray, G.D. & Dewsbury, D.A. (1973) A quantitative description of copulatory behavior in prairie voles (*Microtus ochrogaster*). *Brain Behav. Evol.* **8**, 437–452.
- Gray, G.D. & Dewsbury, D.A. (1975) A quantitative

Limited and complete mating in bank voles

- description of the copulatory behavior of meadow voles (*Microtus pennsylvanicus*). *Anim. Behav.* **23**, 261–267.
- Gray, G.D., Zerynick, M., Davis, H.N. & Dewsbury, D.A.** (1974) Effect of variations in male copulatory behavior on ovulation and implantation in prairie voles, *Microtus ochrogaster*. *Horm. Behav.* **5**, 389–396.
- Gray, G.D., Kenney, A. McM. & Dewsbury, D.A.** (1977) Adaptive significance of the copulatory behavior pattern of male meadow voles (*Microtus pennsylvanicus*) in relation to induction of ovulation and implantation in females. *J. comp. Physiol. Psychol.* **91**, 1308–1319.
- Greenwald, G.S.** (1956) The reproductive cycle of the field mouse (*Microtus californicus*). *J. Mammal.* **37**, 213–222.
- Gustafsson, T.O. & Andersson, C.B.** (1980) Adrenal growth during pregnancy in the bank vole, *Clethrionomys glareolus*: initiation by mating. *Can. J. Zool.* **58**, 1458–1461.
- Gustafsson, T., Andersson, B. & Westlin, L.** (1980) Reproduction in a laboratory colony of bank vole, *Clethrionomys glareolus*. *Can. J. Zool.* **58**, 1016–1021.
- Hasler, J.F.** (1975) A review of reproduction and sexual maturation in the microtine rodents. *The Biologist* **57**, 52–86.
- Jorné-Safriel, O.** (1968) *Some factors affecting the adrenal juxtamedullary zone in the vole, Microtus agrestis, and bank vole, Clethrionomys glareolus*. D. Phil. thesis, University of Oxford.
- Kenney, A. McM. & Dewsbury, D.A.** (1977) Effect of limited mating on the corpora lutea in montane voles, *Microtus montanus*. *J. Reprod. Fert.* **49**, 363–364.
- Kenney, A. McM., Hartung, T.G. & Dewsbury, D.A.** (1979) Copulatory behavior and the initiation of pregnancy in California voles (*Microtus californicus*). *Brain Behav. Evol.* **16**, 176–191.
- Milligan, S.R.** (1974) Social environment and ovulation in the vole, *Microtus agrestis*. *J. Reprod. Fert.* **41**, 35–47.
- Milligan, S.R.** (1975a) Mating, ovulation and corpus luteum function in the vole, *Microtus agrestis*. *J. Reprod. Fert.* **42**, 35–44.
- Milligan, S.R.** (1975b) The copulatory behavior of *Microtus agrestis*. *J. Mammal.* **56**, 220–224.
- Milligan, S.R.** (1976) Correlation of plasma LH and prolactin levels with the fate of the corpus luteum in the vole, *Microtus agrestis*. *J. Reprod. Fert.* **47**, 111–113.
- Milligan, S.R.** (1979) The copulatory pattern of the bank vole (*Clethrionomys glareolus*) and speculation on the role of penile spines. *J. Zool., Lond.* **188**, 279–282.
- Zarrow, M.X. & Clark, J.H.** (1968) Ovulation following vaginal stimulation in a spontaneous ovulator and its implications. *J. Endocr.* **40**, 343–352.

Received 19 May 1981

EFFECT OF DEXAMETHASONE TREATMENT ON ADRENAL HYPERTROPHY
DURING PREGNANCY IN THE BANK VOLE, *CLETHRIONOMYS GLAREOLUS*

C.B. Andersson

Department of Zoology, University of Lund,
S-223 62 Lund, Sweden

Summary. Mating-induced adrenal hypertrophy in female bank voles was not as pronounced in animals treated with dexamethasone for 12 days during pregnancy (25 µg/100 g Bw, twice daily) as in vehicle injected controls. Adrenal weight after dexamethasone injections was, however, higher than in non-pregnant females. The results suggest that adrenal hypertrophy during pregnancy depends, but not exclusively, on ACTH.

INTRODUCTION

In bank voles the adrenal cortex hypertrophies during pregnancy (Jorné-Safriel, 1968; Andersson & Gustafsson, 1980). The enlargement is induced directly by mating without involvement of hormones from the ovaries or the foeto-placental unit (Gustafsson & Andersson, 1980). The mechanism underlying adrenal hypertrophy is thought to be a neuroendocrine reflex related to the one controlling luteal function but separate from that causing ovulation (Andersson & Gustafsson, 1982).

The present paper presents results on the rôle of ACTH to adrenal hypertrophy during pregnancy in the bank vole.

MATERIALS AND METHODS

Laboratory bred bank voles, *Clethrionomys glareolus*, were used (see Gustafsson, Andersson & Westlin, 1980). From weaning at 18 days until the beginning of the experiment, the females were kept in groups of 4-5. At the start of the experiment the animals were aged 2-4 months and were caged singly for at least 2 days before mating. An adult male was introduced into the cage of each female in the morning and the pair was kept under constant observation until mating. Mated females were randomly assigned to one of two treatments, either injections of dexamethasone or of vehicle solution. The first injection was given at mating, after 1-3 intromissions, after which mating was allowed to continue. The male was left with the female for 12 hours. Dexamethasone (Sigma Chemical Company) was given subcutaneously twice daily (08.00 h and 20.00 h) for 12 days in a dose of 25 µg/100 g body weight, dissolved in 0.1 ml saline/20 g bodyweight. Controls received saline according to the same injection schedule. At autopsy on day 12 of pregnancy, the total body weight, weight of paired adrenals and appearance of embryos and corpora lutea (CL) were recorded.

RESULTS AND DISCUSSION

Out of 30 mated bank voles, 15 were found to be pregnant at autopsy. Low fertility seems to be the rule in primiparous microtines (Coutts & Rowlands, 1969; Milligan & MacKinnon, 1976; Andersson & Gustafsson, 1979). Only the pregnant bank voles are considered below.

Adrenal weight was significantly lower in dexamethasone treated females when compared with saline treated controls on day 12 of pregnancy (Table 1). This presumably reflects a dependence of adrenal hypertrophy on ACTH, since dexamethasone has been shown to act as a potent ACTH-blocking agent (Resko, 1969; Piva, Gagliano, Motta & Martini, 1973; Mann, Cost, Jacobson & MacFarland, 1977). It is unlikely that ACTH alone is responsible for adrenal enlargement after mating as adrenal weight is higher in dexamethasone treated animals (Table 1) than in non-pregnant ones (5.72 ± 0.78 ; Andersson, unpublished). Since the dexamethasone dose given has been shown to completely block ACTH secretion (Piva et al., 1973) it is reasonable to assume that other adrenal stimulating agents are present after mating.

Prolactin has been implied to be of importance in the mechanism causing adrenal hypertrophy in the bank vole (Andersson & Gustafsson, 1982). Mann et al. (1977) found dexamethasone to be effective also in blocking prolactin secretion. However, no block to prolactin secretion seems to have occurred in this study since all females had healthy CL (large, red, vascular; Table 1), which in the pregnant bank vole are dependent on prolactin stimulation (Westlin, 1982). Preliminary results have shown that a prolactin blocking agent (bromocriptine) is effective in blocking adrenal growth during pregnancy (Andersson, unpublished). Thus, it appears that adrenocortical hypertrophy is dependent on ACTH and also on at least prolactin.

The development of the embryos were effected in the dexamethasone treated females (Table 1). The embryos were smaller and resorptions were much more common after dexamethasone treatment than after saline injections. The smaller size of the embryos is mirrored by a lower bodyweight of dexamethasone treated females. The adverse effect on embryonic development could be due to either a direct effect of dexamethasone or to a diminished ACTH dependent adrenal secretion, likely to be corticosterone (Andersson & Vawda, 1983). At present it is not possible to separate these effects.

The financial support from the Swedish Natural Science Research Council and the Royal Physiographic Society of Lund is acknowledged.

REFERENCES

- Andersson, C.B. & Gustafsson, T.O. (1979) Delayed implantation in lactating bank voles, *Clethrionomys glareolus*. J. Reprod. Fert. 57: 349-352.
- Andersson, B. & Gustafsson, T. (1980) The adrenal cortex during and after pregnancy in the bank vole *Clethrionomys glareolus*. Gen. Comp. Endocrinol. 40: 343.
- Andersson, C.B. & Gustafsson, T.O. (1982) Effect of limited and complete mating on ovaries and adrenals in bank voles *Clethrionomys glareolus*. J. Reprod. Fert. 64: 431-435.
- Andersson, C.B. & Vawda, A. (1983) Plasma corticosterone and corticosteroid-binding globulin concentrations during pregnancy and pseudopregnancy in the bank vole, *Clethrionomys glareolus*. Submitted to J. Endocrinol.
- Coutts, R.R. & Rowlands, I.W. (1969) The reproductive cycle of the Skomer vole (*Clethrionomys glareolus skomerensis*). J. Zool., Lond. 158: 1-25.
- Gustafsson, T.O. & Andersson, C.B. (1980) Adrenal growth during pregnancy in the bank vole, *Clethrionomys glareolus*: initiation by mating. Can. J. Zool. 58: 1458-1461.
- Gustafsson, T., Andersson, B. & Westlin, L. (1980) Reproduction in a laboratory colony of bank vole, *Clethrionomys glareolus*. Can. J. Zool. 58: 1016-1021.
- Jorné-Safriel, O. (1968) Some factors affecting the adrenal juxtamedullary zone in the vole, *Microtus agrestis*, and bank vole, *Clethrionomys glareolus*. D. Phil. thesis, University of Oxford.
- Mann, D.R., Cost, M.G., Jacobson, C.D. & MacFarland, L.A. (1977) Adrenal gland rhythmicity and pituitary regulation of adrenal steroid secretion. Proc. Soc. Exp. Biol. Med. 156: 441-445.
- Milligan, S.R. & MacKinnon, P.C.B. (1976) Correlation of plasma LH and prolactin levels with the fate of the corpus luteum in the vole, *Microtus agrestis*. J. Reprod. Fert. 47: 111-113.
- Piva, F., Gagliano, P., Motta, M. & Martini, L. (1973) Adrenal progesterone: factors controlling its secretion. Endocrinol. 93: 1178-1184.
- Resko, J.A. (1969) Endocrine control of adrenal progesterone secretion in the ovariectomized rat. Science 164: 70-71.
- Westlin, L.M. (1982) Increased fertility in young primiparous female bank voles, *Clethrionomys glareolus*, treated with prolactin or progesterone after mating. J. Reprod. Fert. 66: 113-115.

Table 1. Effect of dexamethasone treatment after mating on adrenal weight (mean $\pm$ S.E.), embryo size (mean crown-rump length, CRL, $\pm$ S.E.) and appearance of corpora lutea (CL) on day 12 of pregnancy.

	Dexamethasone	Vehicle
Body weight, g	18.2 $\pm$ 0.7	21.6 $\pm$ 1.1 ^a
Adrenal weight, mg	9.96 $\pm$ 1.04	15.04 $\pm$ 1.30 ^b
CRL, mm	7.5 $\pm$ 0.7	10.1 $\pm$ 0.3 ^c
Proportion of females with resorptions	6/8	0/7
Proportion of females with healthy CL	8/8	7/7

^{a-c} significantly different from dexamethasone treated females, Mann-Whitney U-test: ^a P < 0.05; ^b P < 0.02; ^c P < 0.01.

PLASMA CORTICOSTERONE AND CORTICOSTEROID-BINDING GLOBULIN CONCENTRATIONS DURING PREGNANCY AND PSEUDOPREGNANCY IN THE BANK VOLE, *CLETHRIONOMYS GLAREOLUS*

C. B. Andersson

Department of Zoology, University of Lund,
S-223 62 Lund, Sweden

and

A. Vawda, Department of Zoology, University of Durban-
Westville, Private Bag X54001, Durban 4000, South Africa

Summary. Plasma corticosterone and corticosteroid-binding globulin (CBG) concentrations were measured throughout pregnancy and pseudopregnancy and in early lactation in the bank vole. From the non-pregnant value of 120.1 $\mu\text{g}/100\text{ ml}$ plasma, corticosterone levels rose to 171.0 $\mu\text{g}/100\text{ ml}$ in mid-pregnancy. During the second half of pregnancy there was a sharp rise, levels reaching a peak of 398.1 $\mu\text{g}/100\text{ ml}$ on day 18. Following parturition on day 18 there was a fall to 187.8 $\mu\text{g}/100\text{ ml}$ on day 3 of lactation. CBG concentrations rose in a similar way from $5.10 \times 10^{-6}\text{ mol/l}$, but reached a peak value of $158.41 \times 10^{-6}\text{ mol/l}$ on day 15. Throughout pseudo-pregnancy corticosterone levels were about 200 $\mu\text{g}/100\text{ ml}$ and CBG levels remained stable around $35 \times 10^{-6}\text{ mol/l}$. Adrenalectomy on day 15 of pregnancy reduced corticosterone levels to a value not significantly different from that found on day 0.

INTRODUCTION

Plasma corticosteroid levels during pregnancy have been measured in a number of species e.g. in the mouse (Gala & Westphal, 1967; Barlow, Morrison & Sullivan, 1974; Dalle, Giry, Gay & Delost, 1978), the rat (Ogle & Kitay, 1977; Nicholas & Hartmann, 1981), the guinea-pig and the rabbit (Gala & Westphal, 1967). It appears that there is no change or a small increase in corticosteroid levels in the rat and a slight decrease in the rabbit during pregnancy, while a pronounced increase post partum has been reported for both species. In the pregnant mouse and guinea-pig, however, there is a substantial rise in corticosteroid levels with peak values in late pregnancy followed by a sharp decrease a few days before parturition. Elevated plasma corticosteroid levels seem to be associated with increased corticosteroid binding globulin (CBG) capacity (Gala & Westphal, 1967; Nicholas & Hartmann, 1981). Indeed, elevated CBG and corticosteroid levels during pregnancy appear to occur in most species with hemochorial placentation (Seal & Doe, 1967).

In late pregnancy and/or post partum an increase in adrenal size occurs in mice, rats, guinea-pigs and rabbits (see Parkes & Deanesly, 1966, for review). Corticosterone concentrations in the adrenal glands during pregnancy and

lactation have been measured by Dalle et al. (1978) in the mouse, but they found only small changes. In comparison with other rodents, the adrenal glands of microtines (voles and lemmings) increase dramatically in weight during pregnancy and pseudopregnancy (Chitty & Clarke, 1963; Christian & Davis, 1966; Jorné-Safriel, 1968; Hasler & Banks, 1975; Andersson & Gustafsson, 1980). However, the levels of corticosteroids have not been determined in any microtine species. In view of this, CBG capacity and concentrations of corticosterone in plasma and adrenal glands of pregnant and pseudopregnant bank voles were determined.

MATERIALS AND METHODS

Animals and mating procedures

Laboratory bred bank voles, *Clethrionomys glareolus*, kept as described by Gustafsson, Andersson & Westlin (1980) were used. From weaning at 18 days until the start of the experiment at 2-4 months of age, the females were kept in groups of 4-5. They were then caged singly for 2 days before an adult male was introduced. The pair was checked every 1-2 h and the finding of a vaginal plug was taken as evidence of mating (day 0 of pregnancy). The male was then left with the female overnight. If no mating occurred during the day, the male was removed and re-introduced the following day. Females (5-7) were killed between 13:00 and 14:00 h every third day throughout pregnancy and pseudopregnancy (resulting from a mating with a vasectomized male). Six lactating and 5 virgin females were also included.

Collection of samples

Blood samples were collected by cardiac puncture into heparinized syringes under ether anaesthesia. All blood samples were centrifuged at 9000 g for 2.5 min and the plasma removed and stored at -20°C until required for analysis. No more than 90 sec elapsed between the first handling of the cage and the completion of sampling. The right adrenal was removed and stored in 200 μl of absolute alcohol at -20°C until assayed for hormone content.

Surgical methods

Twelve females were adrenalectomized (ADX) or shamadrenalectomized (SHADX) on day 15 of pregnancy and killed 24 h later. Surgery was performed under ether anaesthesia through a single dorsal skin incision and a bilateral lumbar incision. Adrenals were not handled during SHADX. After ADX the animals were given 0.9 % saline to drink. At autopsy the completeness of ADX was checked under a dissecting microscope.

Corticosterone and CBG assays

The concentration of total corticosteroids was determined by competitive protein binding assay using human CBG (Murphy, 1967). Progesterone was removed prior to assay

using hexane extraction (Swain, 1972). Corticosterone was extracted with dichloromethane. The mean extraction efficiency was $61 \pm 3 \%$. The inter- and intra-assay coefficients of variation were 9.1 and 15.4 %, respectively. The sensitivity of the assay, as defined by the smallest amount of steroid significantly different from zero was 300 pg.

Binding protein concentration was determined using the method of Rousseau, Baxter & Tomkins (1972). Endogenous steroids were removed by incubating 10 μ l plasma samples with 800 μ l phosphate buffered saline and 200 μ l dextran coated charcoal (1000 μ g in 400 μ l gelatin phosphate buffer) for 1 h at 37°C. After centrifugation the supernatant was used for assay. The binding protein concentration was determined by Scatchard analysis (Scatchard, 1949).

Statistical analysis

Data were analysed using the Mann-Whitney U-test. All tests were two-tailed.

RESULTS

Pregnancy

Plasma corticosterone concentrations during pregnancy are shown in Text-fig. 1. There was no significant difference in corticosterone levels between day 0 of pregnancy (128.4 ± 19.0 μ g/100 ml) and non-pregnant females of similar age (120.1 ± 32.7 μ g/100 ml). The level of corticosterone rose gradually from day 0 to day 9 (171.0 ± 18.8 μ g/100 ml; $P < 0.01$) and then increased sharply up to day 18 (398.1 ± 90.6 μ g/100 ml; $P < 0.01$). On day 3 of lactation corticosterone levels had fallen to 187.8 ± 35.8 μ g/100 ml, markedly lower than on day 18 of pregnancy ($P < 0.05$) and not significantly different from day 0 of pregnancy.

Concomitant with the rise in plasma corticosterone levels there was an increase in CBG concentrations (Text-fig. 1). Values rose from $8.17 \times 10^{-6} \pm 1.79 \times 10^{-6}$ mol/l on day 0 to $158.41 \times 10^{-6} \pm 18.29 \times 10^{-6}$ mol/l on day 15 and decreased significantly thereafter to reach $42.60 \times 10^{-6} \pm 4.42 \times 10^{-6}$ mol/l on day 3 of lactation ($P < 0.01$).

Adrenal corticosterone levels (Table 1) varied considerably during pregnancy, with low values on day 12 of pregnancy and on day 3 of lactation.

Pseudopregnancy

Concentrations of plasma corticosterone during pseudopregnancy varied only little around a fairly high level (~ 200 μ g/100 ml) sustained for 18 days (Text-fig. 1). At day 21 levels had dropped to 63.6 ± 22.4 μ g/100 ml.

Variations in CBG levels were small after an initial rise from day 0 ($7.10 \times 10^{-6} \pm 2.17 \times 10^{-6}$ mol/l) to day 3 ($36.10 \times 10^{-6} \pm 9.23 \times 10^{-6}$ mol/l; $P < 0.01$; Text-fig. 1).

Adrenalectomy

Adrenalectomy on day 15 of pregnancy reduced corticosterone levels to a value not significantly different from that on day 0 (Table 2). After shamadrenalectomy on day 15 corticosterone concentrations remained high, not different from levels found on days 15 and 18 of pregnancy (Table 2).

DISCUSSION

The present experiment has demonstrated that there is a substantial increase in total corticosterone levels during the second half of pregnancy in the bank vole. This is in agreement with events during pregnancy in the mouse (Gala & Westphal, 1967; Brain & Nowell, 1970; Barlow et al., 1974; Dalle et al., 1978) and the guinea-pig (Gala & Westphal, 1967). However, during the last four days of pregnancy there is a sharp decrease in total corticosterone concentrations in the mouse (Barlow et al., 1974; Dalle et al., 1978) but no significant decrease was seen before lactation in the bank vole.

CBG concentrations increased during pregnancy in the bank vole up to day 15 and then started to decline i.e. CBG concentrations were decreasing during the last three days of pregnancy when total corticosterone levels were still high. This would presumably result in an increase in the level of free corticosterone during three days before parturition in the bank vole. In the mouse, Gala & Westphal (1967) reported a 13-fold increase in CBG-activity on day 18 of pregnancy and according to Barlow et al. (1974) 98.5 % of the corticosterone is protein bound. They still considered the 1.5 % of free corticosterone to be a considerable increase in the concentration of free hormone compared with resting levels in non-pregnant mice. However, the elevated level of free corticosterone in the mouse is not sustained during the final days of pregnancy (Gala & Westphal, 1967; Barlow et al., 1974) as it is in the bank vole.

The changes in plasma corticosterone levels were not mirrored by the adrenal content of this hormone, although a postparturient fall in corticosterone levels was found in both the plasma and in the adrenals. On the other hand, after a sharp increase in plasma corticosterone concentrations between days 9 and 12 of pregnancy, adrenal corticosterone content was low on day 12. These results differ from those reported by Dalle et al. (1978) in the mouse, where after insignificant changes during pregnancy adrenal corticosterone concentrations rose after parturition.

The finding of high plasma corticosterone levels in pseudopregnant bank voles is not unexpected, since there is an adrenal weight increase even during pseudopregnancy, presumably reflecting increased activity (Andersson & Gustafsson, 1980). After an initial increase in CBG levels there was little variation in either corticosterone levels or CBG concentrations; they remained at levels comparable to those found on days 6-9 of pregnancy.

It is probable that the gradually enlarging adrenals are the main source of corticosterone during pregnancy in the bank vole, since adrenalectomy on day 15 resulted in a rapid drop in plasma corticosterone concentrations. Although levels were still high after adrenalectomy they did not differ significantly from non-pregnant or day 0 pregnant animals. It seems as if resting total corticosterone levels are very much higher in the bank vole ($\sim 120 \mu\text{g}/100 \text{ ml}$) than in the mouse ($2\text{--}2.5 \mu\text{g}/100 \text{ ml}$; Barlow et al., 1974). In the mouse the foeto-placental unit secretes corticosterone in addition to the adrenals (Barlow et al., 1974). In view of the result of adrenalectomy this seems unlikely in the bank vole but the corticosterone secreting role of the foeto-placental unit can not be completely ruled out.

The significance of high corticosterone levels during pregnancy is poorly understood. It has been suggested by Gala & Westphal (1967) that an increase in the concentration of free (biologically active) corticosterone may induce lactogenesis in the rat. They based their assumption on their finding of a decline in CBG values towards the end of pregnancy, thus increasing the level of unbound corticosterone (Gala & Westphal, 1965). This was, however, contradicted by Nicholas & Hartmann (1981) who found only small changes in CBG capacity during the last four days of pregnancy. The role of increased levels of corticosterone during pregnancy in the bank vole is being investigated.

I am grateful to Dr S. Milligan, King's College, London, for providing the facilities for the assays. This work was supported by the Swedish Natural Science Research Council and by the Royal Physiographic Society of Lund.

REFERENCES

- Andersson, B. & Gustafsson, T. (1980). The adrenal cortex during and after pregnancy in the bank vole, *Clethrionomys glareolus*. Gen. comp. Endocrinol. 40, 343, Abstr.
- Barlow, S.M., Morrison, P.J. & Sullivan, F.M. (1974). Plasma corticosterone levels during pregnancy in the mouse: the relative contributions of the adrenal glands and foeto-placental units. J. Endocrinol. 60, 473-483.
- Brain, P.F. & Nowell, N.W. (1970). Adrenal function in pregnant and lactating mice. J. Endocrinol. 48, xvii-xviii.
- Chitty, H. & Clarke, J.R. (1963). The growth of the adrenal glands of laboratory and field voles, and changes in it during pregnancy. Can. J. Zool. 41, 1025-1034.
- Christian, J.J. & Davis, D.E. (1966). Adrenal glands in female voles (*Microtus pennsylvanicus*) as related to reproduction and population size. J. Mammal. 47, 1-18.
- Dalle, M., Giry, J., Gay, M. & Delost, P. (1978). Perinatal changes in plasma and adrenal corticosterone and aldosterone concentrations in the mouse. J. Endocrinol. 76, 303-309.

- Gala, R.R. & Westphal, U. (1965). Corticosteroid-binding globulin in the rat: possible role in the initiation of lactation. *Endocrinol.* 76, 1079-1088.
- Gala, R.R. & Westphal, U. (1967). Corticosteroid-binding activity in serum of mouse, rabbit and guinea-pig during pregnancy and lactation: possible involvement in the initiation of lactation. *Acta Endocrinol.* 55, 47-61.
- Gustafsson, T., Andersson, B. & Westlin, L. (1980). Reproduction in a laboratory colony of bank vole, *Clethrionomys glareolus*. *Can. J. Zool.* 58, 1016-1021.
- Hasler, J.F. & Banks, E.M. (1975). Morphological changes in the ovaries and other organs of the collared lemmings (*Dicrostonyx groenlandicus*) during pregnancy and pseudopregnancy. *Can. J. Zool.* 53, 12-18.
- Jorné-Safriel, O. (1968). Some factors affecting the adrenal juxtamedullary zone in the vole, *Microtus agrestis*, and bank vole, *Clethrionomys glareolus*. D. Phil. thesis, University of Oxford.
- Murphy, B.E.P. (1967). Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. *J. Clin. Endocrinol.* 27, 973-990.
- Nicholas, K.R. & Hartmann, P.E. (1981). Progressive changes in plasma progesterone, prolactin and corticosteroid levels during late pregnancy and the initiation of lactose synthesis in the rat. *Aust. J. Biol. Sci.* 34, 445-454.
- Ogle, T.F. & Kitay, J.I. (1977). Ovarian and adrenal steroids during pregnancy and the oestrous cycle in the rat. *J. Endocrinol.* 74, 89-98.
- Parkes, A.S. & Deanesly, R. (1966). Relation between the gonads and the adrenal glands. In *Marshall's Physiology of Reproduction*, vol III, pp 1064-1111. Ed A.S. Parkes. Longmans Green & Co Ltd, London.
- Rousseau, G.G., Baxter, J.D. & Tomkins, G.M. (1972). Glucocorticoid receptors: relations between steroid binding and biological effects. *J. Mol. Biol.* 67, 99-115.
- Seal, U.S. & Doe, R.P. (1967). The role of corticosteroid-binding globulin in mammalian pregnancy. In *Proc. 2nd Int. Congr. Hormonal Steroids*, I.C.S. No. 132 pp 697-706. Eds L. Martini, F. Fraschini & M. Motta. Amsterdam: Excerpta Medica Foundation.
- Scatchard, G. (1949). The attractions of proteins for small molecules and ions. *Annal. N. Y. Acad. Sci.* 51, 660-672.
- Swain, M.C. (1972). The assay of plasma progesterone in women by a simple competitive protein-binding method using guinea-pig serum. *Clin. Chim. Acta* 39, 455-461.

Fig. 1. Plasma corticosterone (Δ) and corticosteroid-binding globulin ($\bullet$) concentrations during pregnancy (—) and pseudopregnancy (.....) in the bank vole. Values are means of 5-7 voles $\pm$ s.e.m.

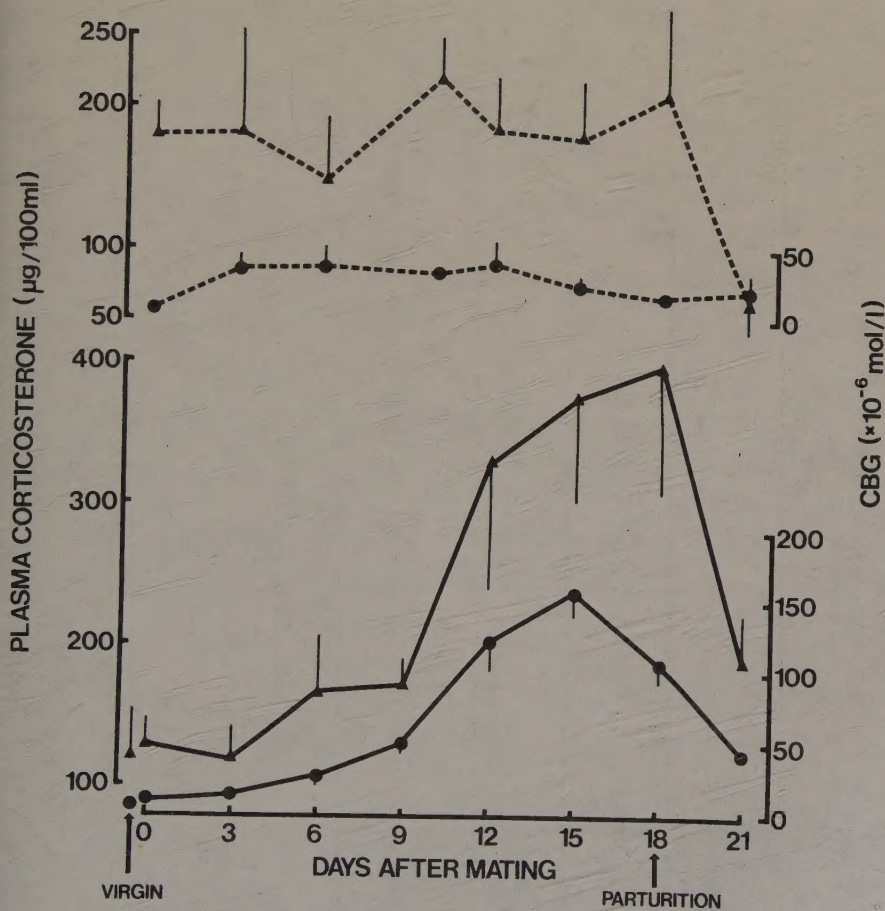


Table 1. Corticosterone concentration in the right adrenal gland on days 6, 12 and 18 of pregnancy and on day 3 of lactation.

Day of pregnancy or lactation	6	12	18	3'
Adrenal corticosterone ($\mu\text{g}/100 \text{ mg}$)	0.34 ± 0.09	0.15 ± 0.06	0.33 ± 0.11	0.13 ± 0.07

Values are mean $\pm$ s.e.m.

Table 2. Plasma corticosterone levels on days 0, 15 and 18 of pregnancy, and on day 16 after adrenalectomy (ADX) or shamadrenalectomy (SHADX) on day 15.

	Pregnant			ADX	SHADX
	0	15	18		
Plasma corticosterone ($\mu\text{g}/100 \text{ ml}$)	128.4 ± 19.0^b	374.9 ± 73.1^a	398.1 ± 90.6^a	149.0 ± 18.7^b	397.5 ± 22.0^a

Values are mean $\pm$ s.e.m.

Means with different superscripts are different ($P < 0.01$)